INTRAMUSCULAR LIPID UTILIZATION DURING EXERCISE: GENDER COMPARISONS



By

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Abstract of Dissertation Presented to the Graduate School of the University Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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The purpose of this study was to evaluate the response of intramyocellular lipid (IMCL) and lipid metabolism indices in men and women in response to submaximal aerobic exercise. Nineteen moderately trained subjects (M=10, W=9) were recruited for the study and were matched for cardiovascular fitness, body composition and dietary intake. Exercise consisted of 60 minutes of stationary cycling at 65% of VO_{2max}. All subjects were given dietary prescriptions for the three days prior to exercise (fat < 30%, carbohydrate 50-60%, and protein 10-15%). All women were eumenoreic and performed their exercise session in the early to mid follicular phase of their menstrual cycle. Blood samples and proton (^1H) nuclear magnetic resonance spectroscopy (MRS) of the vastus lateralis were obtained immediately before and after exercise. Metabolic data, heart rate, and ratings of perceived exertion (RPE) were obtained during exercise. Water was given prior (400 mL) and during exercise (2.5 ml/kg/bw) to maintain hydration. Absolute (kcal) and relative (%) lipid oxidation % was not different between groups as assessed by the respiratory exchange ratio (P=NS). No differences were found in relative carbohydrate

oxidation. Following exercise, IMCL decreased significantly for both men and women ($P<0.05$), with no gender differences (P=NS). Subjects with the highest baseline IMCL had the greatest decrement during exercise ($R=0.93$, $P<0.001$). Following correction for estimated plasma volume changes, significant increases were noted in plasma glycerol and free fatty acids ($P<0.05$). Group (main effects) differences were present for both FFA and glycerol, with female subjects having a greater exercise response. Increased plasma concentrations were noted for norephinephrine, cortisol, and glucagon ($P<0.05$) immediately following exercise, with gender differences for norepinephrine ($P<0.05$). In conclusion, these results suggest that total lipid oxidation, as assessed by RER, was not different between groups. IMCL decreased significantly after exercise for both male and female subjects. Activation of muscle hormone sensitive lipase seems the most plausible mechanism to explain the decrease in IMCL after exercise. Different results from previous studies may be attributed to lack of dietary and/or menstrual control. Future studies should focus on the mechanism involved in IMCL change with exercise.

CHAPTER 1
INTRODUCTION AND REVIEW OF LITERATURE

Introduction and Specific Aims

There are conflicting results regarding the lipid contribution from various lipid storage locations (adipose, plasma, skeletal muscle sources) during exercise when comparing male and female subjects. While some exercise studies find no difference in the percent total lipid (via respiratory exchange ratio (RER)) use between genders, others find that females oxidize more lipid than their male counterparts when exercise intensity is similar (Ruby and Robergs, 1994, Tarnopolsky 2000). Discrepancies in results may be related to physiological differences such as catecholamine response, lipid enzyme activity, or research control variables such as menstrual cycle, exercise type and duration, and subject fitness level. In addition to expired gas analysis, many studies have used either isotopic tracer (for free fatty acid turnover) (Blaak et al. 2000) or skeletal muscle biopsy (for muscle triglyceride (TG)) techniques (Ebeling et al. 1998) to quantify lipid utilization during exercise. These techniques have inherent limitations and do not enable the distinction of lipid stores in different skeletal muscle compartments (intramyocellular (IMCL) and extramyocellular (EMCL)). IMCL is stored as lipid (TG) droplets in the cytoplasm near the mitochondria while EMCL is stored in the interstitial space between myocytes (Boesch et al. 1997). This is important because IMCL contributes significantly to fuel metabolism during moderate intensity exercise, whereas EMCL is thought to be relatively inactive (Boesch et al. 1997). However, there are no published studies that have compared genders with respect to IMCL use during exercise. Studies that examine

IMCL changes between genders may help explain discrepancies in results in gender comparison studies.

We propose to conduct an investigation to compare the effects of exercise on IMCL in males and females. Metabolic and blood data will be collected before and during exercise to help explain the study results.

Hypotheses

The specific hypotheses tested are as follows:

1. **Short-term exercise (60 minutes) will significantly decrease IMCL in both male and female subjects.**

 Although several studies suggest IMCL decreases after exercise in male subjects, there are no published studies that have evaluated IMCL changes in women. Several studies with female subjects have found a greater reliance on fat as a fuel source when compared to men (Ruby and Robergs 1994), although the source of this lipid remains speculative. It is believed that female and male subjects will show a significant decrease in IMCL. The use of ^{1}H-MRS to measure IMCL has shown less variability than repeated muscle biopsy obtainments (Boesch et al. 1997, Wendling et al 1996).

2. **Females will have a greater change in IMCL with exercise compared to males.**

 Perseghin et al. (2001) recently showed IMCL concentrations at rest are elevated in normal healthy female subjects compared to men. Given the results of this study, we hypothesize that female subjects will rely more heavily on IMCL as a fuel source during exercise and show greater reductions in IMCL than their male counterparts. A preferential use of IMCL as a fuel source for female subjects during exercise could help explain differences noted in earlier studies between genders.

3. **Total lipid use will be greater in female subjects, compared to male subjects during exercise of the same duration and intensity.**

 As with IMCL, it is believed that overall fat use, measured by RER is greater in female subjects during exercise. We believe that an increased use of IMCL during exercise may contribute to increased total fat oxidation in female subjects.

4. **Total plasma FFA and glycerol will be greater in female subjects, compared to male subjects following exercise of the same duration and intensity.**

Varying results have been reported between genders for FFA and glycerol during exercise (Ruby and Robergs 1994). We believe that female subjects will mobilize more FFA during exercise compared to male subjects.

5. **Plasma epinephrine and norepinephrine concentrations will be lower in female subjects compared to male subjects following exercise of the same duration and intensity.**

 Results from Ruby and Robergs (1994) suggest that women have a greater sensitivity to catecholamines than men, and have less change in plasma levels during exercise. We believe female subjects will show a lesser elevation of catecholamines following exercise compared to men.

6. **Total carbohydrate oxidation will be lower in female subjects compared to male subjects following exercise of the same duration and intensity.**

 We believe female subjects will have greater lipid oxidation during exercise, and thus a smaller reliance on carbohydrates during exercise.

Review of Literature

The following review will address the effects of acute exercise on lipid metabolism. Given the recent advances in the study of IMCL metabolism, special attention will be placed on ^{1}H-MRS and its application in exercise metabolism studies. The remaining sections will focus on three main areas: 1) a description of lipid fuel metabolism, 2) a discussion of the effects of acute exercise on lipid metabolism, and 3) a comparison of gender differences in lipid metabolism during acute exercise.

It is well recognized that lipids are the primary fuel source at rest (Durstine and Haskell 1994) and with low to moderate intensity exercise (Ruby and Robergs 1994) in healthy subjects. There is some debate, however, whether males and females oxidize similar rates of lipid during exercise (Ruby and Roberts, 1994). For example, some studies find similar rates of fat use during exercise (Costill et al. 1979, Powers et al. 1980, Wallace et al. 1980), while others find greater use by female subjects (Blatchford et al. 1985, Tarnopolsky et al. 1990). The explanation for such discrepancies is unclear, but

may reflect real physiological differences or merely study design issues (Ruby and Robergs 1994, Toth et al. 1998).

In contrast to studies that have examined gender differences in total lipid oxidation, considerably less is known about gender differences in intramyocellular lipid (IMCL) use during exercise. This is important because IMCL contributes significantly to overall oxidative metabolism during moderate intensity exercise (Boesch et al. 1997, Romijn et al. 1993). Differences in IMCL metabolism between genders during exercise may also help explain the inconsistent study results. Traditional techniques used to evaluate IMCL changes with exercise are limited in their ability to distinguish intra and extramyocellular lipid compartments (Boesch 1997, Boesch 1999, Shulman 2000) and results from these studies have been inconsistent.

In an effort to promote a more detailed understanding of IMCL metabolism, a relatively new technique called proton nuclear magnetic resonance spectroscopy (^{1}H-MRS) has gained favor. ^{1}H-MRS can be used to evaluate skeletal muscle lipid non-invasively and has the unique ability to distinguish separate lipid pools. Although a few exercise studies have been completed with male subjects (Boesh et al. 1997, Krssak et al. 2000), no studies to date have been completed with female subjects. Studies with men find a decrease in IMCL after exercise and into recovery (Boesh et al. 1997, Krssak et al. 2000, Rico-Sanz et al. 2000). More work is warranted in this area to clarify gender differences.

Lipid Metabolism

Endogenous triglycerides (TG) represent the largest available fuel source in the human body. The majority of TG is stored as adipose tissue (50,000 - 150,000

kilocalorie (kcal)) (Coyle 1995), while a much smaller supply is available in both skeletal muscle (1800-5000 kilocalories (kcal)) and plasma (Essen et al. 1977, Coyle 1995, Horowitz and Klein 2000). The total amount of stored TG is over 60 times greater than the amount of stored glycogen (Horowitz and Klein 2000). During fasting or prolonged exercise (caloric deficits) an increase in hormone sensitive lipase (HSL-A) activity allows adipose TG to be mobilized as non-esterfied free fatty acid (FFA) and ultimately as fuel (Frayn 1998).

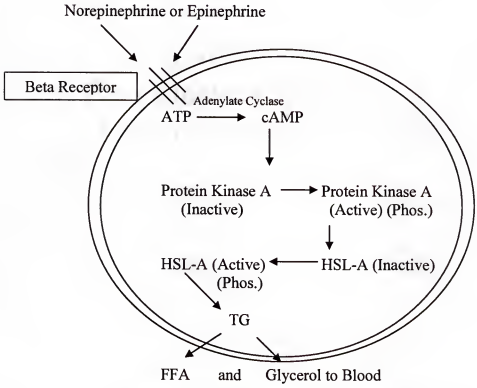
HSL is an 84-kDa protein found in several tissues including adipose, cardiac muscle, and skeletal muscle of mammalian species (Holm et al. 1987, Holm et al. 1989, Holm et al. 2000). The expression of HSL is correlated to fiber type, with higher activity expressed in oxidative fibers (Holm et al. 2000). HSL has very broad substrate specificity and will hydrolyze all acylglycerols: TGs, diglycerides, and monoglycerides and cholesteryl esters (Holm et al. 2000). The major stimulators of HSL-A in adipose cells are plasma catecholamines (epinephrine and norepinephrine). Catecholamines mediate their action through four main adrenergic receptors: β_1, β_2, β_3, and α_2 (Lafontan et al. 1995). The adrenergic receptors are members of a G-protein receptor family and have 7 transmembrane spans (20-28 hydrophobic amino acids), an extracellular amino terminus with glycosylation sites, and an intracellular carboxyl terminus for stabilization of the protein membrane (Premont et al. 1995). The order of receptor affinity for norepinephrine is $\alpha_2 > \beta_1 > \beta_2 > \beta_3$, and for epinephrine is $\alpha_2 > \beta_2 > \beta_1 > \beta_3$ (Lafontan et al. 1995). β_1 receptors mediate the effects of low concentration of catecholamines (Granneman 1995). Less sensitive β_3 receptors require higher concentrations of catecholamines for activation and are a more sustained signal (Granneman 1995).

Microdialysis studies suggest that $\alpha 2$ receptors regulate adipose lipolysis at rest, while β_1 receptors regulate adipose lipolysis mainly during exercise (Arner et al. 1990). However, some work suggests that β_3 receptors may play a role in lipolysis regulation (Emorine et al. 1989).

When catecholamines bind to adrenergic receptors, a signal is tranduced to activate adenylate cyclase through a G protein heterotrimer (See Fig. 1-1). Adenylate cyclase converts adenosine tri-phosphate (ATP) into cyclic adenine monophosphate (cAMP). cAMP binds to the regulatory subunit of protein kinase A, releasing an active catalytic subunit to phosphorylate and activate HSL-A (Holm 2000). Protein kinase A then catalyzes the phosphorylation of HSL-A at 2 sites. Site 2 can also be phosphorylated by Ca^{+2} calmodulin-dependent protein kinase II, AMP-activated protein kinase, and glycogen synthase kinase-4 (Yeaman 1990). Other hormonal activators of HSL-A include growth hormone, cortisol, and thyroid hormone (Frayn 1998).

HSL-A translocates from the cytosol to lipid droplets via a family of lipid droplet associated proteins called perilipins (Blanchette-Mackie et al. 1995). Following translocation, HSL-A hydrolyzes TG into FFA and glycerol for entry into the blood stream (Holm 2000). However, activity of HSL-A can be modified through phosphorylation and dephosphorylation. For example, protein phosphatases 1, 2A and 2C dephosphorylate HSL at site 2 and inhibits it activity (Wood et al. 1993). A second inhibitor of HSL is insulin. Insulin inactivates HSL-A through dephosphorylation at sites 1 and 2 (Stralfors and Honnor 1989). A final important inhibitor of HSL-A is elevated concentrations of plasma fatty acids (Abumrad et al. 1986). High concentrations of plasma fatty acids participate in a feedback mechanism to inhibit activation of HSL

(Abumrad et al. 1986). It has been shown that oleic acid and oleoyl CoA are non-competitive inhibitors of HSL, with 50% inhibition observed at 0.5 and 0.1 uM concentrations (Jepson and Yeaman 1992). These examples illustrate how phosphorylation has a significant impact on lipid metabolism. Liberated glycerol from TG breakdown travels through the blood to the liver and kidney for reesterification (Watford 2000). Liberated FFA is transported through the blood attached to albumin (Frayn 1998) (see Figure 1-2). Transport is mediated by available blood flow in adipose tissue and the availability of albumin.



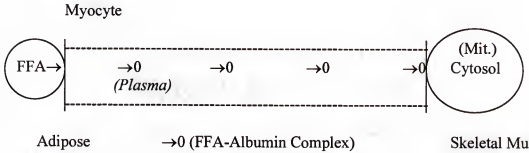
Frayn 1998, Holm et al. 1989, and Lafontan et al. 1995

Figure 1-1. Lipolysis Cascade in White Adipose Tissue

Each albumin molecule has 2-3 high affinity FFA binding sites and several lower affinity sites (Frayn 1998). FFA attached to albumin have several fates and can be 1)

transported to other adipose sites for reesterification, 2) sent to skeletal muscle for storage as TG, or 3) oxidized in skeletal muscle (Oscai et al. 1990). TG stored in very low-density lipoprotein (VLDL-TG) or chylomicron may also circulate in plasma and interact with plasma lipoprotein lipase (LPL) to produce fatty acid for storage in adipose or skeletal muscle, or be used as a fuel source in skeletal muscle (Oscai et al. 1990).

Short and medium chain fatty acids enter muscle and adipose cells by simple diffusion (Bonen et al. 1998). Once inside muscle cells, FFA is transported to the 1) mitochondria or peroxisomes to undergo oxidative degradation, or 2) to the sarcoplasmic reticulum to be esterfied into phospholipid or TG (Van der Vusse et al. 1992). The intracellular translocation of fatty acid is driven by the fatty acid concentration gradient across the cytoplasm, collisional interactions of fatty acid binding proteins within the cell membrane, and the driving force of the total concentration gradient of fatty acid (Van der Vusse and Reneman 1995) (see Figure 1-3).



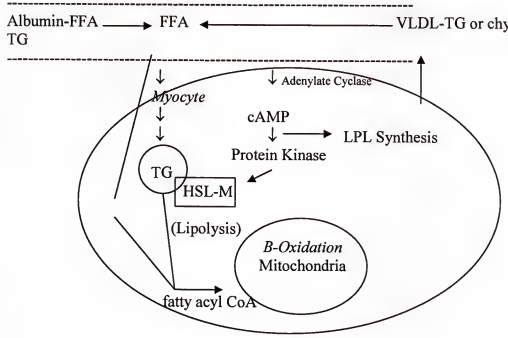
Oscai et al. 1990

Figure 1-2. FFA Transport From Adipose to Plasma and Skeletal Muscle

Long chain fatty acids (LCFA, >12 C) that enter skeletal muscle or adipose stores require a carrier-mediated system (Bonen et al. 1998). This is inconsistent with earlier data that suggested LCFA rapidly transverses the lipid bilayer of the cell membrane by a

diffusional process due to their hydrophobic nature (Bonen et al. 1998). Abumrad et al. (1981) were one of the first to show uptake of LCFA as a saturable process in isolated adipocytes. More recent work has been completed with skeletal muscle verifying this (Bonen et al. 1999).

LCFA transporters are more abundant in more oxidative (red) muscle fibers than glycolytic (white) fibers, suggesting that slow fibers have a greater capacity to utilize lipids than fast fibers (Bonen et al. 1998).



Drawn from Oscai et al. 1990

Figure 1-3. Muscle TG Hydrolysis

To date, two putative fatty acid transporter proteins have been identified. These include the fatty acid binding protein (FABP) and fatty acid transport protein (Isola et al. 1995, Ibrahimi et al. 1996, Schaffer and Lodish, 1994). The amount of fatty acid

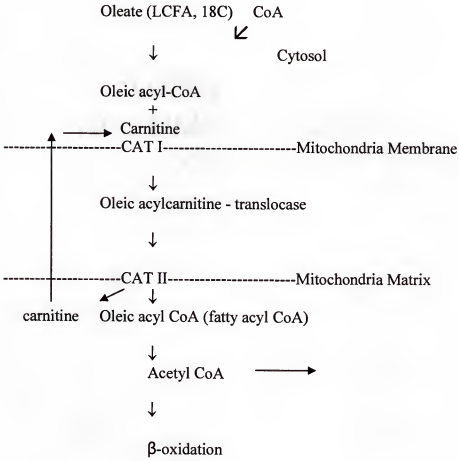
transporter protein expressed is dependent on the rate of fatty acid uptake and/or cellular metabolism (Glatz and Van der Vusse 1996). Therefore, during chronic conditions of increased fatty acid use (i.e., obesity, starvation), greater expression of fatty acid transport protein occurs.

Once FFAs are transported into the cytoplasm, its length influences how it enters the mitochondria of the myocyte. Medium and short chain FFA do not need transporters; and once past the inner mitochondrial membrane, FFA can be ultimately converted into acetyl CoA (Wolfe 1998).

In contrast to short and medium chained fatty acids, LCFA must first bind to carnitine (a reaction catalyzed by the enzyme carnitine palmitoyltransferase I (CPT-1) to enter the mitochondria (Fritz 1959) (see Figure 1-4). The product of this reaction, fatty acylcarnitine, is translocated across the inner mitochondria membrane through the carnitine-acylcarnitine translocase system (Pande 1975). Once LCFA moves across the inner mitochondria membrane, carnitine disassociates and the fatty acid is converted to acetyl CoA (Pande 1975). At this point, acetyl CoA from either short, medium, or LCFA can be used to make ketone bodies, stored as IMCL droplets near the mitochondria, or used in beta-oxidation (Wolfe 1998).

The muscle lipid compartment (total lipid in muscle) represents approximately 70-90% of the FFA entering skeletal muscle (Linder et al. 1976). Lipolysis of intramuscular TG is mediated through the second messenger cAMP pathway (Oscai et al. 1990). As with adipose TG lipolysis, hormonal activation of cAMP through the β_2 receptor is required (Oscai et al. 1990). cAMP is responsible for 2 major physiological responses. The first is the activation of protein kinase through phosphorylation.

Activated protein kinase causes a second phosphorylation to occur at a HSL-(muscle, HSL-M) binding site. HSL-M hydrolyzes TG droplets near the mitochondria yielding FFA and glycerol. However, a recent report by Langfort et al. (2000) suggests that muscle contraction independent of the cAMP pathway may activate HSL-M and break down muscle TG and provide a second means for uptake of fatty acids (Langfort et al. 2000). How contraction activates HSL-M independently of the cAMP pathway is not known.



Redrawn from Wolfe 1998

Figure 1-4. Transport of LCFA Across Mitochondria from Cytosol

Liberated fatty acids become available for oxidation in the mitochondria while glycerol is thought to exit and be transported back to the kidney and liver for

reesterification (Oscai et al. 1990, Langfort et al. 2000). However, some data suggest that a limited amount of glycerol kinase is available in muscle to reesterfy glycerol back into IMCL (Guo and Jensen 1999).

The second function of cAMP is to initiate lipoprotein lipase (LPL) synthesis in the muscle. After synthesis, LPL translocates to the capillary endothelium and is involved in plasma TG hydrolysis (Oscai et al. 1990). This allows a constant supply of fatty acid for oxidation or replenishment of TG stores.

Effects of Exercise on Lipid Metabolism

Exercise can alter both resting concentrations of plasma and skeletal muscle lipid (Durstine and Haskell 1994). However, the contribution of plasma and skeletal muscle lipid to the total oxidative metabolism during exercise is dependent upon a number of factors including exercise intensity and duration, pre-exercise nutrition, and individual fitness levels (Kiens 1998).

Lipid utilized during exercise originates from three different sources: 1) albumin bound FFA in blood plasma, 2) fatty acids from circulating VLDL-TG, and 3) fatty acids from TG located in muscle cell (Kiens 1998). A large portion of the increased availability of fatty acids is provided by lipolysis of TG from adipose sites, which increases 2-3 fold during exercise (Wolfe et al. 1990). This response is primarily mediated through increased β-adrenergic stimulation by catecholamines (Arner et al. 1990). A second reason for increased FFA use during exercise is greater blood flow to exercising skeletal muscle. Increased blood flow in muscle shunts blood flow away from adipose sites and allows greater uptake by skeletal muscle. Greater uptake in skeletal muscle limits the availability of fatty acids that can be reesterfied by adipocytes. Up to a

50% decreased reesterification rate in adipocytes has been observed during moderate intensity exercise (Wolfe et al. 1990).

During low intensity exercise, most of the fatty acid oxidized is derived from plasma FFA (Horowitz and Klein 2000). At light and moderate intensity exercise (25-65% of maximal aerobic capacity (VO_{2max})) plasma concentrations of LCFA increase as exercise duration increases (Kiens 1998). In both animal (Paul 1971) and human studies (Havel et al. 1963) with low exercise intensities there is a correlation between plasma LCFA concentrations and their rate of oxidation. Equal turnover and oxidation of LCFA is evident up to 2 hours into exercise (Coyle 1995). However, more recent work suggests that there is a plateau of LCFA uptake at high plasma concentrations (Kiens et al. 1993). Endurance training has been found to increase LCFA uptake ability (Kiens 1993). For example, Turcotte et al. (1992) found a 60% greater capacity for LCFA uptake in trained skeletal muscle of male subjects when compared to untrained skeletal muscle during a 3-hour exercise session. Kiens et al. (1997) attributed this to increased fatty acid binding protein expression following training.

Several exercise studies have compared genders lipid utilization during submaximal exercise (Blatchford et al. 1985, Davis et al. 2000, Horton et al. 1998, Romijn et al. 2000, Tooth et al. 1998). For example, Horton et al. (1998) had trained subjects complete a 2 hour bicycle ride at 40% VO_{2max}. Female subjects had a significantly greater total lipid oxidation than men (50.9 vs. 43.7%, $P<0.05$). Plasma epinephrine and norepinephrine were significantly higher in men after exercise. The authors attributed the greater lipolytic responses in female subjects to increased catecholamine sensitivity at its hormone receptor. Similar findings were observed in a

recent study by Davis et al. (2000). Moderately trained subjects matched for age, fitness and % body fat completed a 90-minute bicycle ride at 47% of VO_{2max}. Catecholamine responses were elevated in male subjects, but greater total body lipolysis was observed in female subjects. The authors again suggested greater catecholamine sensitivity at the receptor site as a reason for gender differences in lipid use.

Differing results were noted by Romijn et al. (2000). They compared lipid utilization in trained women and men at both 25 and 65% of VO_{2max} during 60-minutes of cycle ergometry. No differences were found between genders for RER, fatty acid oxidation, and turnover. Reasons for differences between studies are not clear, but no control was made for menstrual cycle status, which could have impacted results.

As exercise intensity increases (up to ~65 VO_{2max}), total fat oxidation increases, despite a reduction in plasma FFA turnover (Coyle 1995). The increase is attributed to increased IMCL oxidation and may represent almost half of all fat utilized during exercise (Horowitz and Klein 2000).

Only a few studies have compared lipid utilization between men and women at moderate exercise intensities (Carter et al. 2001, Hardman et al. 1983, Hellstrom et al. 1996, Tarnopolsky et al. 1990). Tarnopolsky et al. (1990) ran moderately trained subjects on a treadmill at 65% of VO_{2max} for 15.5 kilometers. Plasma epinephrine was higher in men, and women had lower RER values and greater total fat oxidation. The authors argued that female subjects utilized a greater amount of IMCL during exercise. However, no measurements of IMCL were made. Several other studies with moderately trained subjects have found female subjects to have greater total body fat oxidation than their male counterparts at similar exercise intensities (Carter et al. 2001, Hellstrom et al.

1996, Hardman et al. 1983). Reasons suggested for unequal lipid use between genders included differences in catecholamine sensitivity and differences in alpha and beta-receptor transduction (Carter et al. 2001, Hellstrom et al. 1996, Hardman et al. 1983).

Contrasting findings of the above-mentioned studies is work by Powers et al. (1980). Subjects completed a 90 minute run at 65% VO_{2max}. Although fat oxidation increased over time, there were no differences between men and women including RER values over time. Again, little information is available to explain discrepancies noted between studies.

As exercise intensity continues to increase, ($\geq$ 70% VO_{2max}) there is a decrease in the total % of lipid oxidized (Romijn et al. 1993). The reason for the decline in lipid use during vigorous exercise is complex and probably related to increased concentrations of malonyl CoA, available carnitine levels, lactate, available glycolytic enzymes, fiber type recruited (Brooks and Mercier 1994, Winder 1998), or decreased availability of fatty acid transporter and CPT substrate (Winder 1998).

There are only a few studies comparing substrate selection between genders at higher exercise intensities ($\geq$ 70% VO_{2max}) (Costill et al. 1979, Friedmann and Kindermann 1989). Costill et al. (1979) found little difference in total fat oxidation in trained male and female subjects following a 60 minute running session at 70% VO_{2max}. Friedman et al. (1989) had trained and untrained subjects complete a 10 kilometer run at either 75 or 80% VO_{2max}. Untrained women had a significantly greater fat oxidation than untrained men. However, trained women did not differ in fat utilization than trained men. These results suggest that training-induced adaptations occurred that eliminated potential gender differences. However, Froberg and Pedersen (1984) found increased fat

oxidation in trained women compared to men following 50 minutes of exercise at 80% of VO_{2max}.

Clearly, additional studies need to be completed at different exercise intensities to clarify if gender differences exist in fuel metabolism. Moreover, the contribution of IMCL to lipid metabolism needs to be clarified and may help explain the observed gender differences in fuel metabolism studies.

In addition to the mentioned studies using mostly metabolic and hormonal data, several muscle biopsy studies have been completed with varying results. These include exercise studies with untrained male and female subjects (Hurley et al. 1986, Kiens et al. 1993, Phillips et al. 1996), trained male subjects (Kiens et al. 1993, Kiens and Richter 1998, Starling et al. 1997, Froberg and Mossfedlt 1971, Staron et al. 1989), and in male subjects with mixed diet types (Wendling et al. 1996). Decreases in skeletal muscle TG concentration following acute exercise have ranged from 10 to 30%. However, some exercise studies find no change or non-significant changes in skeletal muscle TG following exercise as well (Bergman et al. 1999, Jansson and Kaijser 1982, Wendling et al. 1996).

Reasons for inconsistencies among studies may be related to exercise mode, exercise intensity or duration. In addition, muscle fiber type, training status of subjects, pre dietary differences, and variability in acquiring muscle biopsy samples before and after exercise may explain differences among studies. Wendling et al. (1996) has observed differences in muscle TG measurement of 20-26%, suggesting a high degree of measurement error.

Another limitation of the muscle biopsy technique is the inability to distinguish intramyocellular (IMCL) and extra myocellular lipid (EMCL) compartments (Shulman 2000). IMCL is lipid found in the cytoplasm near mitochondria, whereas EMCL is lipid found in the interstitial space between myocytes (Boesch 1997). This is important since IMCL contributes more as a fuel source during rest and exercise, whereas EMCL is relatively inert and is metabolically inactive (Boesch et al. 1997). A final limitation may be related to muscle biopsy volume obtainment. Most biopsy samples are small in volume (i.e., 100 mg), and may not represent entire muscle morphology (Boesch et al. 1999).

Isotopic tracers (typically C labels) are another method used for lipid measurement during exercise that provides information about plasma FFA turnover (Wolfe 1998). However, tracer studies make three assumptions that may have limitations as well. These include the assumption that LCFA entering the muscle cell during exercise are not stored in an intracellular TG pool, but are destined for direct oxidation. A second assumption is that the label is not lost in metabolic pathways. A third assumption is that VLDL-TG does not contribute as a quantitative important fuel for use by muscle during exercise. Arguments by Wolfe et al. (1998) and Kiens et al. (1998) have presented recent work to suggest that 2) and 3) are indeed limiting in tracer studies, and should be considered when evaluating study results.

Given the possible limitations with tracer studies, several reports have been published with exercise and lipid metabolism. Recent work by Coggan et al. (2000) found trained men to have greater overall lipid use at high exercise intensities (75-85% VO_{2max}) than untrained men. The authors concluded that trained men had greater plasma

FFA turnover rate and greater use of intramuscular TG during exercise. Identical results were noted by the same group when exercising subjects at milder intensities (65% VO_{2max}) (Coggan et al. 1995). Kanaley et al. (1995) noted similar findings in FFA turnover and oxidation in moderately trained marathon runners.

However, not all studies report findings of increased FFA turnover in trained subjects (Bergman et al. 1999). Because of inconsistencies among studies, more work is needed before a definitive statement can be made about FFA turnover and use during and following exercise.

A promising new technique for evaluating lipid metabolism in muscle is ^{1}H-MRS. This technique is non-invasive and applies electromagnetic radiation to the nuclei of molecules of interest (i.e., metabolites with H) (Boesch 1997, Szczepaniak et al 1999) (see Appendix A). This technique has been validated with biopsy studies and has very good reproducibility (Boesch 1997, Szczepaniak et al 1999). The few studies that have been published using this technique have occurred over the last 4-5 years. Boesch et al. (1997) were one of the first groups to examine muscle lipid changes with exercise using ^{1}H-MRS. They evaluated one male subject who completed 3 hours of continuous cycling at 70% of VO_{2max}. Spectra were obtained at several time points through 100 hours of recovery. IMCL decreased 40% after exercise compared to baseline ($P<0.01$) and recovery of IMCL was not complete until 40 hrs into recovery. In a second study by Boesch et al. (1999), a subject completed 2 marathon runs while on 2 distinct types of diet (low fat and high fat). While on both diets, IMCL decreased dramatically after exercise, but IMCL recovered more rapidly in the high fat diet trial. Similar reductions for IMCL with either a preexercise high fat or low fat diet were seen after a 2-hour run in

trained and untrained subjects (Decombaz et al. 2001). Subjects on the high fat diet returned to 100% of baseline by 15 hours post exercise, with no difference between trained and untrained subjects (Decombaz et al. 2001). However, both trained and untrained subjects on the low fat diet did not reach 100% recovery by 30 hours post exercise. In a study by Krssak et al. (2000) male subjects completed 25 km of intermittent running at 65-70% of VO_{2max}. They noted a 30% drop in IMCL concentration immediately following exercise. These changes correlated with decreased insulin concentration and elevations in FFA during exercise and recovery. Rico-Sanz et al. (2000) found decreases of 15-30% in IMCL in different muscle types (tibialis, soleus, and gastrocnemius) after a 90-minute run at 64% of VO_{2max} in trained men. Pilot work from our laboratory has shown reduction in IMCL in male subjects following exercise. White et al. (in preparation) exercised healthy male subjects intermittently for 45 minutes and found significant reductions in IMCL immediately following exercise, which remained decreased 1 hour into recovery. The results of these studies suggest that ^{1}H-MRS is a useful method to evaluate IMCL utilization with exercise.

Summary

In summary, these results suggest that lipid metabolism is complex and regulated by several factors. At rest, and during light to moderate exercise, lipid serves as the primary fuel source. During moderate intensity exercise lipid from intramuscular sources is thought to make a significant contribution to oxidation, although little data is available to support this argument.

Several exercise studies comparing substrate selection between men and women have been completed. While some gender studies find similarities in lipid use others

report difference between genders. Studies that find differences suggest women have greater catecholamine sensitivity and greater total lipid oxidation. Physiologic and study design issues may help explain some of the findings of these studies. Moreover, since none of these studies have been able to distinguish lipid compartments, ^{1}H-MRS studies may provide additional information.

CHAPTER 2
METHODS

Experimental Design

Nineteen moderately active male and female subjects were recruited (20-36 y, N=9 M and N=10 F). Subjects made two laboratory visits during the study period. The study period lasted two weeks, and included one screening visit and one exercise trial for the study. During visit one, subjects completed several questionnaires that included informed consent, health risk, diet, physical activity, and menstrual history, had anthropometric measures taken (weight and height, % body fat, waist to hip ratio), a resting blood pressure measurement, and a maximal bicycle exercise test for fitness assessment (VO_{2max}). This information was used to determine subject study inclusion.

Three days prior to the second visit, subjects were prescribed a standard American Heart Diet (50-60% carbohydrate, < 30% fat, 10-15% protein). Food logs were kept during this time to document nutrient intake. During the second visit, subjects completed a 60-minute cycle exercise session at the Department of Radiology's site for Magnetic Resonance studies. Prior to exercise, subjects had a catheter placed in an antecubital vein for serial blood collection during and after exercise. A pre exercise proton (^1H) magnetic resonance (MRS) scan of the vastus lateralis was made to determine intramyocellular (IMCL) content before exercise. Resting metabolic data was obtained during a 10 minute rest period before exercise. Subjects then completed 60-minutes of cycle exercise at 65% of VO_{2max}. During exercise, metabolic and blood (a subset of male and female subjects)

data was obtained. Immediately following exercise, a finale blood sample was collected and a second ^{1}H-MRS scan was acquired from each subject.

Exercise, alcohol and caffeine use was limited 2 days prior to the exercise trial, to eliminate any residual carry over effects. The study design is outlined in Figure 2-1.

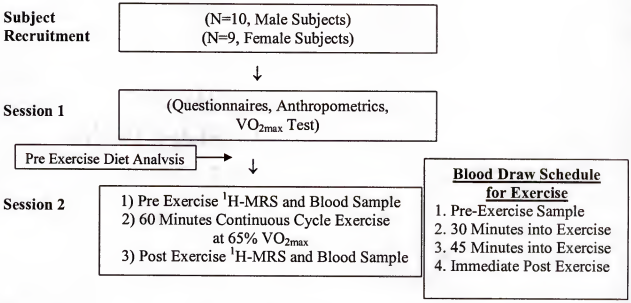


Figure 2-1. Experimental Design

Subjects

Ten male and nine female subjects were recruited from the University of Florida general student population (N=19, 20-36 y). Inclusion criteria included: 1) current participation in an aerobic exercise program (3-5 times per week) for the previous 6 months, 2) not using tobacco products, 3) not taking lipid altering medications, 4) not using contraceptive medications, 5) not having orthopedic limitations that would prevent participation in moderate to vigorous exercise, 6) having normal healthy levels of body fat (men 10-20%, women 15-25%, Powers 2001), 7) not having been diagnosed for coronary artery disease, and 8) having an estimated ventilatory threshold above 65% of

VO_{2max}. To further minimize the confounding effects of differences in fitness, subjects were matched for VO_{2max} (± 5 ml·kg fat free mass (FFM)·min^{-1}). Thus, for each male subject recruited, a female subject was recruited to match, and both subjects had a VO_{2max} within ± 5 ml·kg FFM·min^{-1} of each other. All subjects had a history of weight stability at the time of the study, with no more than a 2-kg weight loss or gain over the 6 months prior to entry. Pre study dietary habits were evaluated by having all subjects' complete two-day dietary logs. Subjects that had large deviations (± 25%) from traditional American Heart type diets (carbohydrate intake 50-60%, fat intake < 30%, and protein intake 10-15%) were excluded as study participants (Lauber and Sheard 2001). Menstrual cycle histories were assessed with questionnaires to ensure female subjects were eumenorreic (normal cycle for previous 6 months) and that they completed their exercise trial during the early to mid follicular phase of their menstrual cycle. This time period was chosen to minimize estrogens impact on lipid metabolism (increased FFA mobilization), normally observed during the luteal phase (Nathan and Chaudhuri 1997). Guidelines as established by the University Florida Institutional Review Board for the use of human subjects were followed and all subjects signed informed consents prior to study entry (Appendix K).

Anthropometric Measures

Body weight and height was determined with a standard physician's scale. Body mass index (BMI) was calculated by dividing body weight (kg) by height (meters squared). Waist-hip ratio was calculated by dividing the waist measurement by the hip measurement.

Body density was estimated using the three skinfold site method of Jackson-Pollock (1978). The average of three measurements was used. Body fat % was calculated with the formula of Brozek et al. (1963).

Maximal Exercise Test

Maximal VO_2 (aerobic fitness) was determined using a modified Åstrand protocol (1986) with a Par O True Max 2400 metabolic cart (Salt Lake City, Utah) and Monark Cycle (Varberg, Sweden). The protocol began at 50 watts (50 RPM) and was increased by 25 watts every minute until VO_{2max} was reached. This test was designed to elicit VO_{2max} in 8-12 minutes. Criteria used for maximal VO_2 was one or more of the following, 1) subject exhaustion, 2) a < 2 ml/kg increase in oxygen consumption with an increase in work rate, 3) a RER greater than or equal to 1.1 (Taylor et al. 1955), and 4) a rating of perceived exertion greater than 17 (Franklin 2000). Respiratory gas variables were measured continuously and include expired gas volume (VE), VO_2, carbon dioxide production (VCO_2) and RER. Analyzers were calibrated with a gas mixture containing known concentrations of carbon dioxide and oxygen before each assessment. Heart rate and blood pressure were measured during each stage of the exercise test.

Ventilatory threshold (VT) was estimated from expired gases acquired during the VO_{2max} test. VT was identified as the breakpoint in the linear increase of expired ventilation (VE) and VO_2 plotted over time (Caizzo et al. 1982).

Exercise Protocol

All subjects completed their exercise session (2nd visit) at the Department of Radiology's 3T site for Clinical Magnetic Resonance studies. Subjects reported in a fasted state (8-10 hours, at 6:00-8:00 P.M., due to magnet availability). Resting

metabolic measurements (10 minutes) were made before exercise, after a short 15 minute rest period. To maintain hydration during exercise, water was provided before and during exercise. Immediately before exercise, 400 ml of water was ingested by each subject (Gisolfi et al. 1992) and during exercise water was given after minutes 30 and 45 (4 ml per kg/body weight at each time point, Ferguson et al. 1998). Each subject completed 60 minutes of cycle exercise at 65 ± 5 % of VO_{2max}. The exercise protocol was continuous and included a five minute cycle warm-up. The exercise protocol was chosen to optimize mobilization and use of IMCL as a fuel source during exercise (Romijn et al. 1993). During exercise, heart rates were measured with a Polar heart rate monitor (Woodbury, NY) and subjects had expired gas measurements at baseline and at 15 minute intervals during exercise. Workloads were adjusted when subjects VO_2 deviated ± 5 % from the prescribed intensity.

Blood Collection

Subjects had a catheter placed in an antecubital vein prior to exercise for the collection of blood samples during exercise. In some cases venipuncture was used when catheter acquisition was not available. Blood samples (10 ml) were collected immediately before and after exercise in EDTA tubes (with a 1mM glutathione additive). A subset of subjects had blood taken during exercise (30 and 45 minutes, M=5, F=3). During exercise, the catheter was kept patent with a small bolus (1-2 cc after each sample collection) of saline. The total volume of blood collected during the study was less than 100 ml per subject. Samples were stored in a cold refrigerator until centrifugation. Following exercise, hematocrit and hemoglobin determination was made on whole blood and the remaining blood was centrifuged into plasma and packed cells. Plasma was

stored at –70 degrees C until assays were completed. Plasma analysis was completed within six months following collection.

Day-to-day variability in blood parameters was minimized by collecting blood samples during the same time of day for each subject.

Dietary Analysis

Subjects were given standard dietary instructions for nutrient intake during the three days prior to the exercise trial. Intake instructions were based on American Heart Association Guidelines (i.e. 50-60% carbohydrate, < 30% fat, 10-15% protein) (Lauber and Sheard 2001). Total kcal intake range recommendations were based on body weight and from estimated resting metabolic rate (Cunningham 1980, BMR= 500 + (LBM x 22)). Information from physical activity questionnaires was also used to aid in the calculation of total kcal intake so that subjects were isocaloric prior to the exercise trial. Food exchange lists with serving sizes were used for nutrient recommendations (Health Management Resources, Boston, MA.). Subjects were asked to complete dietary records for all three days prior to the exercise trial. Alcohol was prohibited for two days prior to exercise and caffeine the day of exercise. Nutrient intake and distribution (total kilocalorie intake, % fat, % carbohydrate, and % fat) was completed using ESHA Nutritional Software Version 7.7 (Salem, OR).

Blood Analysis

Hematocrit percent was determined by the microcapillary tube method. Hemoglobin concentration was determined with the cyanmethemoglobin techniques as described by Drabkin and Austin (1935). Estimated plasma volume changes during

exercise were used to correct post exercise blood metabolite and hormone concentrations (Dill and Costill, 1974).

Plasma glucose, free fatty acids (FFA), glycerol, and triglyceride (TG) concentrations were measured with colorimetric reagent kits (Eagle Diagnostics, DeSoto TX, Sigma Diagnostics, St. Louis, MO, Wako, Richmond, VA.). Blood lactate was analyzed with the Accusport Lactate Analyzer (Boehringer Mannheim, Mannheim, Germany). Plasma catecholamines including epinephrine and norepinephrine were measured using high pressure liquid chromatography (HPLC) as described by Casuson et al. 1982. Cortisol was analyzed using an in house radioimmunoassay (RIA) kit developed by Vanderbilt University (Hormone Assay Core, Nashville, Tenn). Growth hormone (GH) was determined using an RIA using the Nichols Institute Diagnostics kit (San Juan Capistrano, CA) (Hunter et al. 1962). Glucagon was measured with an RIA kit based on the methods of Aguilar-Parada et al. 1969. Insulin was measured with an RIA procedure based on the methods of Wide and Porath 1966. All assays were performed in either duplicate or triplicate and in a single run. The average with in variability of samples were: glucose 8.2%, TG 5.3%, FFA 0.4%, glycerol 6.8%, lactate 5.1%, epinephrine 7.4%, norepinephrine 4.2%, cortisol 7.5%, GH 10.1%, glucagon 4.5%, and insulin 6.3%.

In vivo ^{1}H-MRS

Proton magnetic resonance spectroscopy (^{1}H-MRS) was performed before and after exercise using a 3.0 T whole body scanner (SIGNA-VH2, General Electric, Milwaukee, MI, USA). Localized ^{1}H spectra were obtained using a quadrature driven birdcage knee coil in the tranceive mode. Subjects were placed in the bore of the magnet

in a supine position, with feet first. The anatomical location for the region-of-interest (ROI) was 1/3 the distance from the superior aspect of the patella and iliac crest. This method has been used in previous studies (White et al. 2000). The ROI was identified with a felt marker and the leg was padded to maintain leg and body position in the magnet. The coil was placed at mid-section level of the vastus lateralis of the right leg. Axial scout images were obtained using either a T_1 weighted spin echo (500ms TR, 17ms TE), or a T_1 weighted gradient echo (500ms TR, 15ms TE) sequence. The voxel of 1.5x1.5x1.5 cm^3 was selected at the middle of the vastus lateralis where the skeletal muscle is homogeneous and to avoid obvious fat contamination (Figure 2.2). The magnetic field was homogenized on the water signal from the same voxel. The water signal was suppressed by three consecutive CHESS (*Che*mical *S*hift *S*elective) pulses (Galloway et al. 1987). The water suppressed ^1H signal was then obtained from the voxel using PROBE-SV PRESS (*P*oint-*res*olved *s*pectroscopy, Bottomley 1984) with echo time = 45 or 25 ms, repetition time = 2 sec, 128 average, and 2048 data points collected.

Spectra Fitting

The spectral raw data were apodized by 1.25 Hz line broadening and zero filled after 20Hz high-pass Gaussian convolution filtering. The proton spectra were reconstructed by Fourier transformation and the zero/first order phase correction. Each moiety contents were estimated using a Gaussian and/or Lorentzian fit in the frequency domain. IMCL was identified as peaks at 1.28 ppm and EMCL at 1.4 ppm (Boesch et al. 1997). The % changes of IMCL and EMCL were obtained by normalization using the water and choline contents from the same voxel. In addition, IMCL values are presented in arbitrary units as represented by the total area under the curve. Repeated

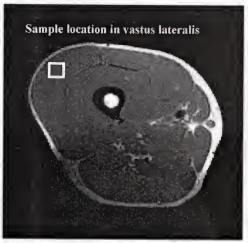


Figure 2.2
Voxel

measurements on a single subject found a variability of 6% from trial to trial (6 scans).

Data Analysis

Subject descriptive comparisons were made with student t-tests. Spectra information, blood metabolite information, dietary information, and plasma volume data were analyzed with group x time repeated measures analysis of variance (ANOVA). When significant main effects were observed, post hoc analysis was completed with student t-tests to determine location of pair-wise differences. Pearson correlations were performed with different variables of interest. An alpha level $P<0.05$ was considered significant. All statistics were performed with SPSS (Version 11.0, Chicago, Il).

Sample Size

The primary dependent variable in the current study was IMCL. Sample size calculations from Dawson-Saunders and Trapp (1990) were used to estimate the number of subjects required. Sample size estimates were based on previous literature (Decombaz et al. 2001). The results of the power calculation indicate fourteen subjects (n=7 each group) would provide a power of 80% at an alpha level of 0.05 to detect differences in

IMCL. In anticipation of subject dropout, 9 subjects per group were recruited. (See Power calculation, Appendix C).

CHAPTER 3
RESULTS

The purpose of this study was to evaluate gender differences in intramyocellular lipid (IMCL) and indices of lipid metabolism in male and female subjects in response to 60-minutes of submaximal cycle exercise. Moderately trained subjects from the local community were recruited. Male and female subjects were matched for cardiovascular fitness (± 5 $ml \cdot kg \cdot min^{-1}$) and body fat % (normal healthy ranges). Descriptive characteristics are summarized in Table 3-1. Male subjects had significantly less body fat, body mass indexes (BMI) and waist/hip ratios ($P<0.05$). However, cardiovascular fitness was similar between groups when expressed relative to total body weight and relative to fat free mass (FFM) (P=NS). Subjects were screened for dietary intake (two-day analysis) prior to study inclusion. Dietary intake and nutrient consumption inclusion criteria was based upon guidelines established by the American Heart Association (carbohydrate intake 50-60%, protein intake 10-15%, and fat intake < 30% of total nutrient intake) (Lauber and Sheard 2001). Dietary analysis for the three days prior to exercise revealed no group differences for total kcal intake when expressed kcal/kg body weight (P=NS). There were also no group differences for dietary nutrient composition (% fat, carbohydrate, protein, P=NS) (Table 3-2). Overall dietary intake for subjects was similar to young active adults of the United State population (Borrud et al. 2002).

All subjects completed 60 minutes of cycle exercise at 65% of VO_{2max}. Exercise trials were performed in the evening (6-8 PM) following an 8-10 hour fast. A standard

Table 3-1. Subject Characteristics

Variable	Males (N=10)	Females (N=9)	P Value (M vs. F)
Age (y)	27.4 ± 1.5	27.2 ± 4.1	0.95
Weight (kg)	79.4 ± 2.7	65.5 ± 3.3	<0.01*
Height (cm)	174.7 ± 1.7	169.3 ± 2.1	0.09
Waist (cm)	75.4 ± 2.4	82.8 ± 1.8	0.03*
Hip (cm)	98.3 ± 1.8	101.1 ± 1.8	0.32
W/H Ratio	0.76 ± 0.01	0.82 ± 0.01	<0.01*
BMI	22.8 ± 1.0	25.8 ± 0.7	0.04*
BF (%)	12.9 ± 0.7	20.0 ± 1.1	<0.01*
VO_{2max} ($ml \cdot kg \cdot min^{-1}$)	45.0 ± 1.6	41.5 ± 2.8	0.31
VO_{2max} ($ml \cdot kg \cdot FFM \cdot min^{-1}$)	51.6 ± 1.5	51.9 ± 3.4	0.95

W/H = waist to hip ratio, BMI = body mass index, BF = body fat, VO_{2max} = maximal oxygen uptake, FFM = fat free mass
Mean ± SE

400 ml bolus of water was given 1-2 hours before exercise to maintain hydration. In addition to pre exercise hydration, 4 ml/kg/body weight of water was given to each subject at minutes 30 and 45 of exercise (Ferguson et al. 1998). Thus, each subject consumed approximately 1 liter of total fluid immediately before and during exercise. Average weight loss following exercise was 1.6 ± 0.3 pounds for male subjects and 2.1 ± 0.3 pounds for female subjects (P=NS between groups). Immediately following exercise

Table 3-3. Metabolic and RPE Data During Exercise

Variable	Gender	Pre Exercise	15 min	30 min	45 min	60 min	P Value	
VO_2	M	3.7 ± 0.07	29.2 ± 1.07[1]	29.3 ± 0.97[1]	29.7 ± 0.88[1]	30.6 ± 0.84[1]	Interaction	0.11
($ml \cdot kg^{-1} \cdot min^{-1}$)	F	3.6 ± 0.08	26.8 ± 1.82[1]	26.9 ± 1.88[1]	26.4 ± 1.59[1]	26.9 ± 1.63[1]	Group	<0.001*
							Time	<0.001*
RER	M	0.83 ± 0.01	0.97 ± 0.01[1]	0.93 ± 0.01[1]	0.92 ± 0.01[1]	0.90 ± 0.01[1]	Interaction	0.33
(ratio)	F	0.84 ± 0.02	0.97 ± 0.02[1]	0.92 ± 0.01[1]	0.90 ± 0.02[1]	0.89 ± 0.01[1]	Group	<0.001*
							Time	<0.001*
Heart Rate	M	69 ± 3	146 ± 4[1]	152 ± 3[1]	156 ± 2[1]	158 ± 3[1]	Interaction	0.83
(bpm)	F	73 ± 3	149 ± 4[1]	154 ± 4[1]	157 ± 5[1]	156 ± 4[1]	Group	<0.001*
							Time	<0.001*
RPE	M	----------	13.3 ± 0.4	14.1 ± 0.3[2]	14.7 ± 0.4[2]	15.0 ± 0.4[2]	Interaction	0.10
	F	----------	13.0 ± 0.4	13.3 ± 0.4	13.7 ± 0.4[2]	13.9 ± 0.4[2, 3]	Group	<0.001*
							Time	<0.001*

[1]P<0.05, compared to pre
[2]P<0.05, compared to 15 min
[3]P<0.05, Males vs Females
M, Males, F, Females, VO_2, oxygen consumption per minute, RER, Respiratory exchange ratio, bpm, beats per minute, RPE, rating of perceived exertion
Mean ± SE

(Main data graphs in Appendix B)

Table 3-2. Nutritional Intake

	Day 1				Day 2				Day 3			
	Tot Kcal	% Cho	% Fat	% Protein	Tot Kcal	% Cho	% Fat	% Protein	Tot Kcal	% Cho	% Fat	% Protein
Males	2753 ± 256	61 ± 2	21 ± 3	18 ± 4	2297 ± 212	60 ± 2	21 ± 2	19 ± 2	995 ± 119[1]	58 ± 9	31 ± 8	12 ± 1
Females	2072 ± 445	64 ± 3	24 ± 3	12 ± 3	2175 ± 247	65 ± 3	21 ± 4	14 ± 2	1078 ± 331[1]	65 ± 5	18 ± 5	17 ± 3

[1]P<0.05, compared to Day 1
Tot Kcal, Total Kilocalories, Cho, Carbohydrates
Mean ± SE

P Values:	Tot Kcal	% Cho	% Fat	% Protein
Interaction	0.14	0.41	0.32	0.97
Group	<0.001*	0.45	0.28	0.10
Time	<0.001*	0.97	0.71	0.39

there was a 13-14% reduction in plasma volume in both male and female subjects ($P<0.05$, Table 3-5). Plasma volume changes were used to correct for the effects of hemoconcentration in plasma metabolite and hormonal concentrations.

During exercise, male subjects expended 695 ± 25 kcal, and female subjects expended 522 ± 41 kcal ($P=0.003$). Kcal expenditure per kg FFM during the 60-minute exercise session was 10.0 kcal for both male and female subjects respectively ($P>0.05$).

Average oxygen uptake (VO_2,) respiratory exchange ratio (RER), heart rate and rating of perceived exertion (RPE) during exercise are listed in table 3-3. Over time VO_2, heart rate, and RPE increased ($P<0.001$). RER initially increased at minute 15 of exercise and then decreased slowly through the remaining 45 minutes. Group differences (main effects) were observed for VO_2, RER, and RPE ($P<0.001$). Post-hoc analysis showed only gender differences for RPE at minute 60 of exercise ($P<0.05$), with males having a higher RPE at this time point than females.

Based on RER data, estimated exercise caloric expenditure of total lipid was 22.5 ± 2.3% for male and 26.4 ± 4.1% for female subjects (P=NS). There were no gender differences in absolute lipid oxidation (157 kcal (male) versus 130 kcal (female), P=NS) during exercise. Similar results were found when expressing exercise lipid oxidation per kg FFM (2.27 kcal/min (male) versus 2.46 kcal/min, (female), P=NS). Assuming that protein made little contribution to fuel metabolism during exercise, total carbohydrate use was 77% and 74% for male and female subjects, respectively (P=NS). However, male subjects oxidized a greater absolute amount of carbohydrate (538 kcal) than female subjects (391 kcal) during exercise ($P<0.05$).

Table 3-4. Intramyocellular Lipid Changes

Gender	Variable	Pre	Imm Post	P Value	
M	IMCL	$1.6 \times 10^5 \pm 26337$	$1.2 \times 10^5 \pm 22131$[1]	Interaction	0.03*
F	(AU)	$3.3 \times 10^5 \pm 39362$	$1.7 \times 10^5 \pm 22430$[1]	Group	0.09
				Time	<0.01*
M	IMCL % Δ	100 ± 0	-18.6 ± 8.6[1]	Interaction	0.03*
F	(AU)	100 ± 0	-43.7 ± 9.6[1]	Group	0.50
				Time	<0.001*
M	IMCL/Choline	2.9 ± 0.6	1.7 ± 0.3[1]	Interaction	0.17
F	(AU)	3.2 ± 0.7	1.6 ± 0.3[1]	Group	0.10
				Time	0.002*
M	IMCL/Choline % Δ	100 ± 0	-30.1 ± 10.9[1]	Interaction	0.07
F	(AU)	100 ± 0	-48.8 ± 6.9[1]	Group	0.50
				Time	<0.001*
M	IMCl/Water	$4.6 \times 10^{-3} \pm 0.0001$	$2.8 \times 10^{-3} \pm 0.0004$[1]	Interaction	0.84
F	(AU)	$4.9 \times 10^{-3} \pm 0.0008$	$2.5 \times 10^{-3} \pm 0.0004$[1]	Group	0.38
				Time	0.01*
M	IMCL/Water % Δ	100 ± 0	-27.7 ± 10.1[1]	Interaction	0.18
F	(AU/Water)	100 ± 0	-42.4 ± 9.5[1]	Group	0.50
				Time	<0.001*

[1] $P<0.05$, imm post vs pre

M = Male, F = Female, IMCL = Intramyocellular Lipid, Imm = Immediate, AU = Arbitrary Units
N = 8 Male and N = 7 Female
Mean ± SE

Intramyocellular lipid (IMCL, muscle lipid located near mitochondria) (Boesch et al. 1999) results are presented in Table 3-4. Data are expressed in arbitrary units (AU) (total area under the curve), AU/muscle choline, and AU/muscle water. Typical relative comparisons are made with either AU/water, AU/creatine, or AU/choline when these metabolites remain constant (Boesh et al.1997, 1999). Muscle choline and muscle water levels were similar between pre and post exercise (P=NS) and were used to normalize IMCL peak area (Boesch et al. 1997). Since significant changes were observed for muscle creatine after exercise ($P<0.05$), it was not used to normalize IMCL data.

Both male and female subjects exhibited significant decreases in IMCL following exercise ($P<0.01$). This finding was present regardless of how IMCL was expressed (i.e., AU, IMCL/water, IMCL/choline) ($P<0.01$). The IMCL decrement following exercise was approximately 25% and 45% for male and female subjects, respectively ($P<0.01$).

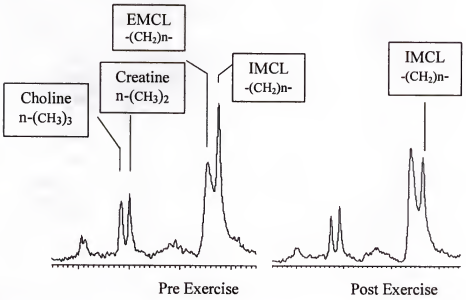


Figure 3-1. Pre and Post Spectra Exercise Spectra of a Male Subject

Table 3-5. Plasma Volume Variables

Gender	Variable	Pre	Imm Post	P Value	
M	HCT	42.8 ± 0.7	46.5 ± 0.6[1, 2]	Interaction	0.79
F	(%)	37.4 ± 1.0	40.9 ± 0.6[1]	Group	<0.01*
				Time	<0.001*
M	Hb	14.3 ± 0.5	15.6 ± 0.6[1]	Interaction	0.64
F	(g/dl)	13.2 ± 0.4	14.5 ± 0.7[1]	Group	<0.01*
				Time	<0.01*
M	PV% Δ	100 ± 0	-13.9 ± 3.5[1]	Interaction	0.86
F		100 ± 0	-13.0 ± 3.1[1]	Group	0.50
				Time	<0.001*

[1]P<0.05, imm post vs pre
[2]P<0.05, Males vs Females at imm post
HCT = Hematocrit, Hb = Hemoglobin, PV% Δ = Plasma volume % change
N = 8 Males and N = 8 Females
Mean ± SE

No gender differences were observed in IMCL changes with exercise. Similar results where found pre to post exercise when expressing IMCL changes per kg FFM (P<0.05). Again, there were no gender differences (males, 5.3 x 10^{-3} ± 0.0001, pre, versus 3.1 x 10^{-3} ± 0.0004, post) (females, 6.2 x 10^{-3} ± 0.001 pre, versus 3.1 x 10^{-3} ± 0.0004, post) (P=NS).

Extramyocellular lipid (EMCL) (lipid between muscle fibers (Boesch et al 1999) did not change following exercise for either group (P=NS). Figure 3-1 illustrates typical spectra obtained from a male subject before and after exercise.

When correlating IMCL changes with other physiological indices, subjects with the highest baseline IMCL or the highest body fat % showed the greatest decrease in IMCL following exercise (R=0.93, P<0.001 and R=0.69, P<0.01, Table 3-10). In

Table 3-6. Blood Metabolites - Uncorrected for Plasma Volume Shifts

Gender	Variable	Pre	Post	P Value	
M	TG	123 ± 5	134 ± 4	Interaction	0.03*
F	(mg/dl)	132 ± 5	129 ± 4	Group	<0.01*
				Time	0.20
M	Glycerol	10.2 ± 1.9	18.9 ± 1.7[1]	Interaction	0.28
F	(mg/dl)	7.8 ± 1.4	20.1 ± 3.3[1]	Group	0.04*
				Time	<0.001*
M	FFA	0.34 ± 0.05	0.61 ± 0.07[1]	Interaction	0.52
F	(mEq/L)	0.31 ± 0.06	0.63 ± 0.08[1]	Group	0.01*
				Time	<0.001*
M	Glycerol/ FFA	31.0 ± 4.3	33.0 ± 4.1	Interaction	0.87
F	Ratio	31.9 ± 8.6	32.0 ± 3.5	Group	0.43
				Time	0.84
M	Glucose	88 ± 8	108 ± 12[1]	Interaction	0.88
F	(mg/dl)	88 ± 9	105 ± 12[1]	Group	0.02*
				Time	0.01*
M	Lactate	2.1 ± 0.2	3.7 ± 0.1[1,2]	Interaction	0.03*
F	(mmol/l)	1.6 ± 0.1	2.5 ± 0.1[1]	Group	0.29
				Time	<0.001*

[1]P<0.05, imm post vs. pre
[2]P<0.05, Males vs. Females imm post
TG = Triglyceride, FFA = Free fatty acids
N = 8 Males and N = 8 Females
Mean ± SE

addition, subjects with the greatest catecholamine increase during exercise had the least amount of change in IMCL following exercise.

Blood metabolite and hormonal data are listed in Tables 3-6, 3-7, 3-8, and 3-9. Immediately following exercise there was a significant increase in plasma glycerol, free

Table 3-7. Blood Metabolites - Corrected for Plasma Volume Shifts

Gender	Variable	Pre	Post	P Value	
M	TG	123 ± 5	116 ± 6	Interaction	0.24
F	(mg/dl)	132 ± 5	112 ± 4[1]	Group	0.36
				Time	0.01*
M	Glycerol	10.2 ± 1.9	16.2 ± 1.6[1]	Interaction	0.24
F	(mg/dl)	7.8 ± 1.4	17.3 ± 1.1[1]	Group	0.04*
				Time	<0.001*
M	FFA	0.34 ± 0.05	0.51 ± 0.05[1]	Interaction	0.28
F	(mEq/L)	0.31 ± 0.06	0.54 ± 0.05[1]	Group	0.001*
				Time	<0.001*
M	Glycerol/FFA	31.0 ± 4.3	33.0 ± 4.1	Interaction	0.87
F	Ratio	31.9 ± 8.6	32.0 ± 3.5	Group	0.43
				Time	0.84
M	Glucose	88 ± 8	91 ± 9	Interaction	0.87
F	(mg/dl)	88 ± 9	90 ± 8	Group	0.45
				Time	0.52
M	Lactate	2.1 ± 0.2	3.1 ± 0.2[1]	Interaction	0.21
F	(mmol/l)	1.6 ± 0.1	2.2 ± 0.1[1]	Group	0.40
				Time	<0.001*

[1]P<0.05, imm post vs. pre
TG = Triglyceride, FFA = Free fatty acids
N = 8 Males and N = 8 Females
Mean ± SE

(Main data graphs in Appendix B)

fatty acid (FFA), glucose, and lactate concentration for both groups compared to pre exercise (P<0.05). When corrected for percent plasma volume changes associated with exercise, significant increases were found for glycerol, FFA, and lactate for both groups (P<0.001). Following exercise, glycerol and FFA increased 59% and 50% for male subjects and 121% and 74% for female subjects, respectively (P<0.05). Changes in

Table 3-8. Blood Hormones - Uncorrected for Plasma Volume Shifts

Gender	Variable	Pre	Imm Post	P Value	
M	Epinephrine	64 ± 21	96 ± 18[1]	Interaction	0.15
F	(pg/ml)	20 ± 3	52 ± 10[1]	Group	0.04*
				Time	0.04*
M	Norepinephrine	303 ± 72	721 ± 174[1]	Interaction	0.28
F	(pg/ml)	256 ± 61	718 ± 125[1]	Group	<0.01*
				Time	<0.001*
M	Cortisol	10.1 ± 3.3	20.0 ± 4.0[1]	Interaction	0.73
F	(ug/dl)	8.5 ± 2.1	17.0 ± 1.7[1]	Group	0.16
				Time	<0.01*
M	GH	5.6 ± 3.1	16.4 ± 4.3[1]	Interaction	0.99
F	(pg/ml)	3.7 ± 1.1	10.5 ± 3.9[1]	Group	0.04*
				Time	0.02*
M	Glucagon	64.5 ± 6.7	93.3 ± 11.3[1]	Interaction	0.94
F	(pg/ml)	47.2 ± 4.5	73.3 ± 7.6[1]	Group	<0.01*
				Time	<0.001*
M	Insulin	2.6 ± 0.6	2.3 ± 0.6	Interaction	0.61
F	(uU/ml)	3.1 ± 1.1	2.5 ± 0.5	Group	0.07
				Time	0.52

[1]P<0.05, imm post vs pre
GH = Growth Hormone
N = 8 M and N = 8 F
Mean ± SE

glucose were no longer significant when adjusted for plasma volume changes (P=NS). Although significant group (main effect) differences were present for glycerol and FFA, there were no significant post-hoc group-comparisons at any time points (P=NS). This was due to the variability in responses of subjects.

Table 3-9. Blood Hormones - Corrected for Plasma Volume Shifts

Gender	Variable	Pre	Imm Post	P Value	
M	Epinephrine	64 ± 21	83 ± 16	Interaction	0.66
F	(pg/ml)	20 ± 3	45 ± 8	Group	0.13
				Time	0.13
M	Norepinephrine	303 ± 72	613 ± 153[1]	Interaction	0.28
F	(pg/ml)	256 ± 61	630 ± 129[1]	Group	0.01*
				Time	<0.01*
M	Cortisol	10.1 ± 3.3	23.0 ± 4.5[1]	Interaction	0.81
F	(ug/dl)	8.5 ± 2.1	20.1 ± 2.3[1]	Group	0.24
				Time	0.01*
M	GH	5.6 ± 3.1	13.8 ± 3.5	Interaction	0.97
F	(pg/ml)	3.7 ± 1.1	9.6 ± 3.9	Group	0.11
				Time	0.06
M	Glucagon	64.5 ± 6.7	80.3 ± 10.6[1]	Interaction	0.75
F	(pg/ml)	47.2 ± 4.5	63.7 ± 7.4[1]	Group	0.02*
				Time	0.02*
M	Insulin	2.6 ± 0.6	1.9 ± 0.4	Interaction	0.60
F	(uU/ml)	3.1 ± 1.1	2.1 ± 0.5	Group	0.04*
				Time	0.19

[1]P<0.05, imm post vs pre
GH = Growth Hormone
N = 8 Male and N = 8 Female
Mean ± SE

(Main data graphs in Appendix B)

Significant pre to post exercise (time main effect) changes were found for plasma TG (P<0.05), but post hoc analysis showed that only female subjects had a significant drop in plasma TG (-15%) following exercise (P<0.01).

Table 3-10. Correlation Matrix

		IMCL (AU) Δ	IMCL (AU)/Water Δ	IMCL (AU)/Chol Δ
VO_{2max} (relative)	r=	-0.50*	-0.27	-0.34
	P=	0.05	0.32	0.19
VO_{2max} (rel/FFM)	r=	-0.36	-0.22	-0.29
	P=	0.18	0.41	0.36
VO_{2max} (absolute)	r=	-0.59*	-0.15	-0.32
	P=	0.02	0.59	0.23
Pre IMCL	r=	0.86*	0.93*	0.90*
	P=	<0.001	<0.001	<0.001
% BF	r=	0.69*	0.28	0.50*
	P=	<0.01	0.28	0.04
BMI	r=	-0.04	0.28	0.15
	P=	0.88	0.28	0.58
W/H Ratio	r=	-0.26	0.02	0.08
	P=	0.33	0.93	0.77
Δ FFA	r=	-0.17	-0.07	-0.51
	P=	0.60	0.83	0.09
Δ Norepin	r=	-0.55	-0.70*	-0.84*
	P=	0.10	0.03	<0.01
Δ Epinephrine	r=	-0.31	-0.71*	-0.73*
	P=	0.33	<0.01	<0.01

IMCL = Intramyocellular Lipid, Chol = choline, VO_{2max} = Maximal cardiorespiratory fitness, rel = relative, % BF = % body fat, BMI = body mass index, W/H Ratio = waist to hip ratio, FFA = free fatty acid, norepin = norepinephrine
*P<0.05

Results for plasma metabolites (glycerol, FFA, lactate) collected during exercise were similar to post exercise changes. For example, plasma glycerol increased at both minutes 30 (males, 11.7 ± 2.4, females, 21.2 ± 15.3 mg/dl) and 45 (males, 13.1 ± 2.3,

females, 20.1 ± 10.5 mg/dl) of exercise for male and female subjects compared to baseline ($P<0.05$). There were no gender differences in plasma FFA or glycerol during exercise (P=NS). However, this subset reflected subjects who had significant responses during exercise and may not be representive of each group as a whole.

Several plasma hormones were significantly increased immediately following exercise for both groups, including epinephrine, norepinephrine, cortisol, glucagon, and growth hormone (GH) ($P<0.05$, time main effect). When corrected for plasma volume changes, significant increases were noted for norepinephrine, cortisol, and glucagon ($P<0.05$), but not GH or epinephrine (P=NS). Changes in plasma norepinephrine immediately after exercise represented an increase of 180% and 138% from baseline for male and female subjects, respectively. Cortisol increased by 98% and 112% in male and female subjects immediately after exercise compared to baseline. Group (main effect) differences were present for norepinephrine and glucagon. Post hoc analysis did not reveal any pair wise differences between genders at any time point for norepinephrine or glucagon (P=NS).

When expressing norepinephrine results per kg of FFM, male subjects had an increase in plasma norepinephrine 6.0 pg/kg FFM immediately following exercise. Female subjects increased an average of 8.8 pg/kg FFM ($P<0.05$). However, when expressing norepinephrine increase per kg of fat mass, there was little difference between male and female subjects (30.3 pg/kg versus 28.5 pg/kg, P=NS). Similar trends were found for epinephrine when expressing increases in epinephrine per kg of FFM and kg of fat mass.

During exercise, plasma hormonal responses were similar to those found immediately following exercise. For example, plasma norepinephrine increased at both minutes 30 (males, 646 ± 137 pg/dl, females, 660 ± 240) and 45 (males, 902 ± 213 pg/ml, females, 687 ± 163) of exercise for male and female subjects compared to baseline ($P<0.05$, time main effect). Post hoc analysis revealed gender differences at minute 30 of exercise for norepinephrine. However, this subset reflected subjects who had significant responses during exercise and may not be representive as a whole.

The only plasma hormone that decreased during exercise was insulin. Although decreases in insulin were not significant from pre to post exercise (P=NS), there were significant group differences present ($P<0.05$). Female subjects showed a greater reduction in insulin than male subjects.

In summary, we compared the effects of 60 minutes of aerobic cycle exercise in male and female subjects on IMCL and multiple indices of lipid metabolism. Subjects were matched for fitness, physical activity, and relative body composition. Dietary intake was controlled for the three days prior to exercise. Metabolic, blood, and skeletal indecises were measured before, during, and after exercise. RER, heart rate, VO_2, and RPE increased significantly with exercise. There were no group differences in relative (%) or absolute (total kcal) lipid use as quantified by RER. Significant decreases were found for IMLC immediately after exercise, with no gender differences observed. Subjects with higher baseline IMCL showed the greatest IMCL change following exercise. Significant increases during exercise were found for plasma glycerol, FFA, and lactate in both groups. Significant increases were found for FFA and glycerol immediately following exercise for both groups. FFA and glycerol increased greater for

female subjects. Female subjects exhibited a significant decrease in plasma TG immediately following exercise, while male subjects did not. Both groups had significant increases for plasma norepinephrine, cortisol, and glucagon following exercise. Group differences were observed for norepinephrine and glucagon. Group differences were found for plasma insulin, although pre to post exercise decrements were not significant.

CHAPTER 4
DISCUSSION

The purpose of the current study was to evaluate the responses intramyocellular lipid (IMCL) and other indices of lipid metabolism in men and women in response to 60-minutes of submaximal cycle exercise. The following discussion will describe the study results and further explain the study findings in relation to gender differences in lipid metabolism. A summary and suggestions for future research are included.

To ensure homogeneity, male and female subjects were matched for VO_{2max} (ml/kg fat free mass (FFM)), physical activity status, dietary habits, and relative body composition (body fat % range). Female subjects were tested during the early to mid follicular phase of the menstrual cycle to avoid the confounding effects of elevated estrogen levels on substrate selection during exercise (Nathan and Chaudhuri 1997). Subjects were screened for diet intake prior to study inclusion and were given dietary instructions for the three days prior to the exercise trial to help control for the confounding effects of pre dietary intake on study parameters. All subjects completed 60 minutes of stationary cycle exercise at 65% VO_{2max}. Water was given before and during exercise to maintain hydration.

Metabolic and Cardiovascular Results

During exercise, significant increases in oxygen uptake (VO_2), respiratory exchange ratio (RER), and heart rate were observed for both men and women. These were expected findings. RER (VCO_2/VO_2) was used to quantify total substrate oxidation (lipid versus carbohydrate use) before and during exercise. RER increased during the

first 15 minutes of exercise reflecting an increase in ventilation (Whipp and Ward 1998). Over time, RER decreased (0.90 and 0.89 for both genders at minute 60) reflecting an increase in total lipid oxidation and a relative decrease in total carbohydrate oxidation as exercise progressed. Both absolute (157 kcal vs. 130 kcal) and relative (26% vs. 23%) lipid use, as indicated by RER, were similar between groups. There were no differences in relative carbohydrate use (74% vs. 77%) during exercise, but male subjects oxidized a greater absolute amount of carbohydrate (538 kcal vs. 391 kcal) during exercise, because of their greater body mass. When expressing carbohydrate oxidized per kg FFM, there were little differences between male and female subjects (7.8 kg/FFM versus 7.5 kg/FFM). To ensure that RER values were a valid measurement of substrate use, plasma lactate concentrations were measured during and immediately following exercise. Group lactate means at all time points for both groups were below 4 mmol/L (estimated lactate threshold) supporting the use of RER to estimate substrate use during exercise (Svedenhag 1992).

Exercise studies comparing substrate utilization between genders have found varying results. Wallace and colleagues (1980) did not find any gender differences in lipid use when using RER for quantification during 120 minutes of treadmill running at 70% of VO_{2max}. Powers et al. (1980) also found no differences in the RER of moderately trained male and female subjects who were matched for VO_{2max} and training mileage, during a 90-minute treadmill run at 65% VO_{2max}. Romijn et al. (2000) compared lipid use in trained female and male subjects at 25 and 65% of VO_{2max} during 60 minutes of cycling. No gender differences were observed for RER, fatty acid oxidation, or free fatty acid (FFA) turnover during exercise. In a more recent study, Roepstorff et al. (2002) did

not find any gender differences in lipid use (RER, leg respiratory quotient, FFA with tracer) in subjects who completed a 90 minute bicycle ride at 58% VO_{2max}. Subjects were matched for VO_{2max} (ml/kg/FFM), physical activity levels, training history, and had similar pre-exercise diets. Female subjects were tested in the mid-follicular phase of the menstrual cycle when estrogen levels were lowest. Although there were no differences in metabolic and tracer data, the authors concluded that very low-density lipoprotein triglyceride (TG) and/or TG located between muscle fibers may have contributed significantly as energy sources during exercise for male subjects.

In contrast to these studies, many others have found gender differences in total lipid use during exercise. Blatchford et al. (1985) exercised subjects for 90 minutes on a treadmill at 35% VO_{2max}. Female subjects had lower RER values at minutes 45 and 90 of exercise, suggesting greater lipid oxidation. Tarnopolsky et al. (1990) had subjects complete 90-minutes of cycle ergometry at 63% of VO_{2max}. Throughout exercise female subjects had lower RER values than male subjects, and significantly lower blood glucose and muscle glycogen concentrations after exercise. The authors concluded that there were gender differences in lipid metabolism during exercise. Several recent studies have found similar results. Carter et al. (2001), Davis et al. (2000), Horton et al. (1998), and Toth et al. (1998) found gender differences for RER in studies with exercise intensities ranging from 40-60% of VO_{2max} and exercise durations ranging from 30 minutes to 2 hours in untrained and trained subjects. RER values were significantly lower for female subjects in these studies and the authors concluded that female subjects had a greater ability to oxidize lipid during exercise than men. In addition, many of these studies found differences in plasma hormonal responses (greater increases for male subjects) and

suggested that female subjects may have greater sensitivity to hormonal (epinephrine and norepinephrine) induced lipid oxidation.

Plasma Lipid Marker Results

Although research using RER to quantify substrate (carbohydrate and lipid) use provides valuable information, this method does not enable the source of lipid oxidation to be determined. Lipids may be mobilized from adipose, plasma, and skeletal muscle sources during exercise (Durstine and Haskell 1994). We found plasma markers of lipid oxidation (FFA and glycerol) increased during and immediately following exercise for both groups. Female subjects increased FFA and glycerol concentrations 74% and 121% from baseline values immediately after exercise. Male subjects increased FFA and glycerol concentrations 50% and 59% from baseline respectively. There were overall gender (group main effect) differences for both FFA and glycerol, although post hoc analysis did not find any significant pair-wise comparisons.

Plasma glycerol concentrations have been used as an estimate of whole body subcutaneous adipose lipolysis (Havel 1965, Havel et al.1963). Although plasma levels do not solely reflect the release of glycerol from adipose stores (i.e, doe not take into account substrate turnover), plasma concentrations provide a reasonable estimate of adipose tissue lipolysis. If we accept plasma glycerol as an estimate of adipose lipolysis, female subjects had 2-fold % increase with exercise compared to male subjects, a similar finding recently reported with untrained men and women (Hellstrom et al. 1996, Mittendorfer et al. 2001).

Exercise studies making gender comparisons of plasma FFA and glycerol have yielded mixed results. While some studies find increases in plasma glycerol/and or FFA

for female subjects (Blatchford et al.1985, Davis et al 2000, Hellstrom et al. 1996) others do not (Horton et al. 1998, Tarnopolsky et al. 1990, Toth et al. 1998). Reasons for differences between studies are unclear, but may be related to exercise protocol, lack of menstrual control, and poor control of pre exercise dietary habits.

Mechanisms that may help explain increased adipose tissue lipolysis and greater plasma levels of FFA and glycerol, include 1) increased mobilization of FFA from adipose tissue TG stores and 2) the breakdown of VLDL-TG in plasma (Durstine and Haskill 1994).

Pre-Post Exercise Hormonal Results

The most prominent mechanism cited for increased adipose lipolysis is increased hormonal induced mobilization. FFA from adipose tissue TG is mobilized following hormonal interaction with β_1 receptors on adipocytes during exercise (Arner et al. 1990). The most potent of the lipid mobilizing hormones are the catecholamines (Hellstrom et al. 1996, Lafontan et al. 1995). Norepinephrine is thought to have greater affinity for adipose β_1 receptors than epinephrine during exercise (Arner et al. 1990). When catecholamines bind to β_1 adrenergic receptors, cyclic amine monophosphate (cAMP) initiates a cascade of events resulting in the phosphorylation of adipose hormone sensitive lipase (HSL-A) (Care 1998). Once phosphorylated, HSL-A induces the mobilization of fatty acids from adipose triglyceride stores (Care 1998). Hydrolysis of adipose triglyceride allows the release of fatty acids and glycerol into blood where they are available for uptake in other tissues (Holm 2000). Other moderate hormonal activators of HSL-A include growth hormone, cortisol, and thyroid hormone (Frayn 1998).

In the current study, several hormones that regulate FFA mobilization from adipose tissue increased during exercise. Significant increases (time main effect) were observed for plasma norepinephrine and cortisol immediately after exercise. There were gender (group main effect) differences for norepinephrine, with female subjects showing greater increases compared to male subjects. Although it is impossible to determine which hormone had the greatest effect on fatty acid release from adipocytes, catecholamines are thought to have the greatest affect on adipose tissue lipolysis (Lafontan et al. 1995).

We also observed that total fat mass was positively correlated with changes in plasma norepinephrine ($r=0.56$, $P<0.05$) for female subjects. These data suggest that total fat mass, may be important regulator in lipid oxidation between genders (Hellstrom et al. 1996, Tarnopolsky et al. 1990). The potential mechanism responsible for adipose increased catecholamine activity during exercise is unclear, but warrants further study.

In contrast to lipid mobilizing hormones, other hormones, such as insulin have counter regulatory effects for adipose tissue lipolysis (Davis 2000). Following exercise, plasma insulin concentration was decreased for both male and female subjects ($P>0.05$), with significant group differences ($P<0.05$). Female subjects had a greater decrease than male subjects. Suppression of insulin secretion during exercise occurs through increased sympathetic activity (alpha adrenergic activation in the pancreas, Wahrenberg et al. 1991) and was expected. Results of earlier gender studies comparing insulin's response to exercise show either decreases or no change in plasma insulin (Friedmann 1989, Davis et al. 2000, Tarnopolsky et al. 1990, Horton et al. 1998). Suppression of insulin secretion has been observed with greater FFA use (Wasserman et al. 1989). Given that plasma

FFA were elevated during exercise, RER decreased during exercise, and insulin decreased, our data was similar to reported literature (Wasserman et al. 1989).

Besides catecholamine mobilization, a second contributor to increased plasma glycerol and FFA during exercise is the increased hydrolysis of plasma very low-density lipoprotein triglyceride (TG) into FFA. Hydrolysis occurs following plasma TG's interaction with lipoprotein lipase (LPL) (Durstine and Haskell 1994). We observed significant time main effects for plasma TG, with female subjects showing a significant decrease (-15%). Although some argument has been made for the importance of FFA use from plasma TG during exercise (Steffsen et al. 2002), FFA from this source is probably more important to replenish muscle TG stores several hours after exercise cessation (Oscai et al. 1990). Several studies have suggested that plasma TG provides approximately 5-10% of the total lipid used during moderate intensity exercise (Oscai et al. 1990, Kiens et al. 1998), but its contribution is thought to be minimal compared to either adipose or skeletal muscle sources.

Although the most likely explanation for increased adipose tissue lipolysis, as evidenced by plasma markers, was an increase in hormonal mobilization of fatty acids, the difference between genders may not be explained solely by differences in adiposity or HSL-A. Our data does not allow us to preclude other contributing factors such as 1) gender differences in alpha adrenergic activation (Hellstrom et al. 1996), 2) differences in fatty acid reesterification rates of released fatty acids (Wolfe et al. 1998), and 3) differences in adipose tissue blood flow transporting fatty acids into the plasma (Hellstrom et al. 1996). Future studies are needed to determine factors contributing to fatty acid mobilization from adipose tissue, particularly during exercise.

Intramyocellular Lipid Results

Once fatty acid is mobilized from adipose stores, or VLDL-TG FFA is made available, fatty acid may enter the skeletal muscle for oxidation or storage as intramyocellular lipid (IMCL) (Wolfe 1998). The major finding of this study was a significant decrease in IMCL immediately following exercise for both male and female subjects. Early published studies have used skeletal muscle biopsy techniques for IMCL measurement. This technique has been criticized for its high degree of variability on repeated measurements from the same muscle location (20-25%) and subject discomfort (Shulman 2000, Wendling et al. 1996). A strength of our study was the use of a non-invasive technique, proton (^{1}H) nuclear magnetic resonance spectroscopy (MRS) to measure IMCL. ^{1}H-MRS has the ability to distinguish two separate lipid compartments in muscle, including IMCL and extramyocellular lipid (EMCL) (Boesch et. al 1997). Biopsy techniques do not allow the distinction of these two compartments (Shulman 2000). This is important because IMCL is thought to be more metabolically active than EMCL (Boesch et al. 1997, Boesch et al. 1999). To our knowledge, this is the first study to use ^{1}H-MRS for IMCL measurements for gender comparisons during exercise. This technique has low variability on repeated measurements of the same muscle site (6% in the current study) and has been validated for measurement of IMCL (Boesch 1997, Szczepaniak et al 1999).

The magnitude of decrease in IMCL after exercise in our study averaged approximately 25% for male subjects and 45% for female subjects, depending on IMCL peak area quantitation (AU, AU/water, AU/choline). Although female subjects decreased IMCL more than male subjects, the difference between groups was not statistically

significant. These results suggest that male subjects had a greater reliance on plasma FFA than female subjects, although future tracer studies should test this hypothesis. Similar results were observed when expressing IMCL change per kg/FFM. While some subjects had large decreases in IMCL immediately after exercise, others showed very little change, potentially explaining a lack of difference between groups. Our data is consistent with a recent study by Larson-Meyer et al. (2002). The authors had a group of well-trained women complete a long treadmill run at 67% VO_{2max}. They observed an average of 25% reduction in IMCL in the soleus muscle immediately after exercise, but found a large variation in IMCL changes (0% to 50%) immediately after exercise.

Although only a few studies have used ^{1}H-MRs to evaluate IMCL during exercise, our results are consistent with published reports. The majority of these studies have used well-trained subjects using prolonged (90 or more minutes) running protocols. In a group of trained male distance runners, Rico-Sanz et al. (2000) found significant decreases in IMCL in the tibialis anterior (32%) and soleus (19%) muscle following a 90-minute treadmill run at 64% of VO_{2max}. The authors suggested that differences in oxidative capacity of the muscles accounted for the differences in lipid decrements. Brectel et al. (2001) examined IMCL changes in a group of well-trained male runners following a half and full marathon run. IMCL decreased significantly from 10-57% depending on exercise duration and muscle (tibialis anterior and soleus) examined. Krssak et al. (2000) also examined IMCL changes in a group of trained runners following several repeated discontinuous bouts of running (mean 25 km) to exhaustion. Soleus muscle IMCL decreased 33% and returned to 83% of baseline value 20 hours into recovery. Data from our lab, found decreases in IMCL of vastus lateralis muscle (38%)

in a group of very fit males immediately after a 45 minute interval cycling session (White et al., in review). Sixty minutes into recovery IMCL was still significantly decreased, suggesting little or no recovery of IMCL within the hour following exercise. Finally, Decombaz et al. (2001) examined the effects of 2 hours of running at 50% VO_{2max} in both trained and untrained subjects. Subjects exhibited a 22-26% drop in IMCL (tibialus anterior muscle) immediately after exercise regardless of training status. The authors suggested that increased muscle hormone sensitive lipase (HSL-M) and decreased malonyl-CoA contributed to the decreased IMCL levels.

Although there were no exercise induced gender differences for IMCL decrements, we observed that IMCL changes following exercise were correlated with baseline levels. Thus, those subjects who had the greatest baseline values for IMCL showed the greatest decrease in IMCL following exercise. A similar finding was found by Larsen-Meyer (2002) for female subjects following prolonged moderate intensity exercise. They found IMCL change after exercise to be positively correlated with IMCL baseline levels in a group of well trained female runners.

Inconsistent results have also been observed in exercise studies that have measured IMCL with biopsy techniques. Several studies have found reductions (20-40%) during prolonged running or cycling at exercise intensities between 55-75% VO_{2max} (Boesch et al. 1997, Essen 1977, Essen et al. 1977, Hurley et al.1986) and even greater reductions (42-75%) during ultra endurance competitions (Froberg and Mossfeldt 1971, Lithell 1979, Staron et al. 1989). Although no comparative data between male and female subjects during exercise using ^{1}H-MRS have been reported, a recent study by Steffensen et al. (2002) using muscle biopsy techniques found gender differences in

muscle TG (IMCL) responses after a 90-minute cycle ride. Female subjects were tested during the mid follicular phase of their menstrual cycle and matched for fitness with male subjects. Female subjects decreased muscle TG approximately 25% following exercise, whereas no changes were noted for male subjects. The authors concluded that gender differences existed between subjects and that female subjects used more IMCL than male subjects during exercise. However, other studies have found no changes in IMCL with exercise sessions lasting between 25 minutes (Kaijser 1982) and 2 hours (Bergman 1999, Jansson and Kiens et al. 1993).

Reasons for discrepancies between study (^{1}H-MRS and biopsy) findings are unclear, and may be related to 1) technique measurement error, 2) differences in fiber type and oxidative capacity of tissues studied, 3) pre-exercise dietary intake, 4) exercise mode and intensity, and 5) training status of the subjects.

For example, in a recent study by Howald et al. (2002), the authors compared IMCL measurements between ^{1}H-MRS and biopsy techniques. The authors suggested that lipid volume area between four large muscle cells (EMCL) was approximately 800x the volume area of IMCL in the same four large muscle cell regions. The authors suggested that it was unlikely that biopsy samples were collected without contamination from EMCL sources. These data suggest that studies using biopsy techniques to examine skeletal muscle lipid should be interpreted with caution.

IMCL Change Mechanisms

Mechanisms that help explain IMCL decrements during exercise are speculative, and will be discussed individually. Major regulators of IMCL mobilization and use include the activity of muscle hormone sensitive lipase (HSL-M), transport of fatty acyl

CoA into the mitochondria, availability of beta oxidative enzymes (impacted by muscle fiber type), and reesterification of fatty acids in the myocyte prior to their uptake into the mitochondria (Winder 1998).

Although minimal information is available regarding HSL-M in response to exercise, it is likely that increased HSL-M activity contributed to the availability of muscle fatty acids for oxidation. Immunobloting techniques (Holm 1987) and Northern blotting have found protein and mRNA in muscle similar to that found with HSL-A (Holm 1988). In adipose tissue, the activity of HSL-A (via a cAMP cascade) is regulated by sympathetic and other hormonal activity (Hales et al. 1978). Although skeletal muscle receptors are of the β_2 subtype, compared to the β_1 found in adipose tissue (Liggert et al. 1988), it is speculated that the same initiators that activate HSL-A activate HSL-M. Potential initiators of this cascade include catecholamines, cortisol, and growth hormone (GH) (Holm et al. 2000).

Few studies have been completed that have examined HSL-M changes at rest or during exercise. Peters et al. (1998) demonstrated increased HSL-M protein and muscle TG breakdown following epinephrine administration in isolated slow twitch muscle fibers (soleus) during resting conditions. The authors used a pulse chase labeling technique and concluded that epinephrine infusion activated β_2 sympathetic activity, resulting in muscle TG hydrolysis.

Kjaer et al. (2000) exercised adrenalectomised human subjects for 45 minutes at 70% VO_{2max} on a cycle ergometer. Subjects then completed an additional 15 minutes of cycling at 86% of VO_{2max} and half the subjects received an epinephrine infusion and the other half did not and served as control subjects. Biopsy samples showed increased HSL-

M activity and protein expression immediately after exercise for the epinephrine infusion group. The authors concluded that epinephrine contributed to increased HSL-M during exercise.

In our study, several plasma hormones increased during exercise, providing a potential stimulus for increased HSL-M activity. Increases were noted for norepinephrine, epinephrine, cortisol, and GH, although gender differences were only observed for norepinephrine. To our knowledge, no studies have made gender comparisons of sympathetic activation (via hormone induction) of isolated skeletal muscle fatty acid mobilization (from IMCL sources). Future studies in this area could provide valuable information on the role different hormones play in mobilizing fatty acids from skeletal muscle sources during exercise. Furthermore, studies focused in this area could provide additional information for lipid storage health problems such as Type II diabetes and myopathy.

Besides hormonal regulation of HSL-M, muscle contraction may activate HSL-M through an independent mechanism. Langfort et al. (2000) repeated electrically stimulated (200-ms trains of 100 Hz, impulse duration 0.2 ms, 25 V) rat soleus muscle for 60 minutes. HSL-M was increased in the first minute of stimulation. The authors suggested that contraction induced HSL-M activation occurred through direct phosphorylation of protein kinase A in a calcium++ calmodulin type pathway. If this pathway exists, HSL-M may be activated by dual mechanism (hormonal activation and muscle contraction) (Langfort et al. 2000). Further work is needed to describe the synergistic or independent effect of these mechanisms.

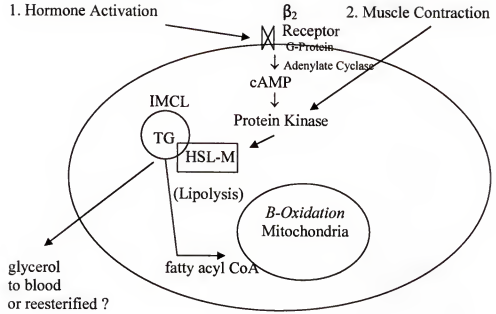


Figure 4.1. Dual Activation of HSL-M in Skeletal Muscle

(Langfort et al. 2000)

A second point of regulation of IMCL (fatty acid mobilization) use during exercise is the uptake of fatty acyl CoA into the mitochondria. FFA from plasma enter the muscle cell through either simple diffusion and/or though fatty acid transport proteins (Wolfe 1998). Fatty acids are also available through hydrolysis of stored IMCL. Fatty acids from either source can interact with fatty acyl CoA synthetase to form fatty acyl CoA. The rate of FFA transport into the cell and availability of fatty acid from IMCL controls the rate of fatty acyl CoA formation (Wolfe 1998). Once long chained fatty acyl CoA is formed, it exits the cytoplasm and enters the mitochondria through the carnitine palmitoyl-transferase I and II transport system (CPT-I and II transport system) (Saggerson et al. 1992). Availability of fatty acyl CoA synthetase, Coenzyme A, and

CPT-I dictate fatty acyl CoA uptake (Winder 1998). Most important of these is the availability of CPT-I (Winder 1998).

At rest, trained subjects have higher mitochondria CPT-I compared to untrained subjects, and males have similar CPT-I levels as females (Berthon et al. 1998). However, muscle fiber type is an important determinant of CPT-I activity. Slow twitch fibers have greater quantities of CPT-I and a greater ability to transport fatty acyl CoA into the mitochondria than fast twitch fibers (Winder 1998). Acute aerobic exercise has little impact on CPT-I levels in trained subjects (Winder 1998). The rate-limiting step for the CPT-I transport system is the interaction of CPT-I with malonyl CoA. Malonyl CoA is a product of malonyl and co enzyme A (Winder 1998). Malonyl CoA is a competitive inhibitor of fatty acyl CoA at the CPT-I binding site of carnitine. Anything that alters the activity of malonyl CoA can have a significant effect on fatty acycl CoA uptake into the mitochondria.

Odland et al. (2000) found a decrease in malonyl CoA following acute exercise. The authors concluded that a decrease in malonyl CoA freed up more binding domains on CPT-I and allowed more fatty acyl CoA to be transport into the mitochondria (Figure 4.2) (Winder 1998). Muscle contraction may decrease malonyl CoA through an AMP-activated protein kinase cascade (Winder 1998). Thus, low malonyl CoA is considered desirable to maximize lipid use during exercise.

A second regulator of CPT-I that may limit the availability of fatty acyl CoA uptake is the presence of acetylcarnitine. Free acetyl groups from the citric acid cycle have the ability to bind carnitine and limit the amount of free carnitine available for interaction with the CPT-I enzyme. This complex slows fatty acyl CoA transport into the

mitochondria (Winder 1998). Hiatt et al. (1989) has shown that muscle acetylcarnitine increases with moderate intensity exercise (50% VO_{2max} for 60 minutes) and is correlated with a reduction in free (66%) and total (19%) carnitine. Unfortunately, there are no published exercise studies that have made gender comparisons for muscle acetylcarnitine and its relationship to substrate selection.

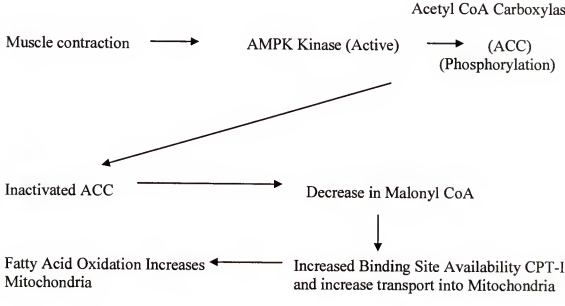


Figure 4.2 Increased fatty acyl CoA uptake through malonyl CoA inhibition (Winder 1998)

In the current study, seven subjects (3 male and 4 female) exhibited a measurable post exercise spectra peak at 2.13 ppm; although the area integrated under the curve was not statistically significant compared to baseline (P=NS). Kreis et al. (1999) and White et al. (2000) have suggested that this peak represents acetylcarnitine. If this is the case, than several subjects exhibited increased acetylcarnitine with exercise, possibly contributing to the study findings. An increase in acetylcarnitine would potentially decrease free

carnitine, and limit fatty acyl CoA transport into the mitochondria. In the seven subjects who exhibited this peak, there was a negative correlation ($r=-0.83$, $P<0.05$) with peak area and plasma FFA increase. Thus, those subjects with the least FFA increase with exercise, had the greatest increase in muscle acetylcarnitine. This was an unexpected finding, and it is tempting to speculate that increased acetylcarnitine concentration feedbacks and limits additional FFA mobilization from adipose tissue into plasma. However, we are unaware of any studies that have examined this question and additional work is needed to clarify the importance of this finding.

A third regulator of IMCL use is the activity and concentration of beta oxidative enzymes. As fatty acyl CoA from IMCL enters into the inner mitochondrial matrix, available beta oxidative enzymes impact how rapidly oxidation occurs (Brooks and Mercier 1994) and ultimately controls the rate of IMCL oxidation. Acute exercise does not appear to affect beta oxidative enzyme activity and concentration (3-hydroxylacyl-CoA dehydrogenase, etc.) (Zonderland et al. 1999, Lawler et al. 1993), and may be more dependent on fiber type. Slow twitch fibers have a greater mitochondria volume and greater oxidative capacity than fast twitch fibers (Pette and Spamer 1986). We used the vastus lateralis for IMCL measurement in our study. The vastus lateralis muscle is approximately 40-50% slow and 40-50% fast twitch in fiber makeup (Simoneau and Bouchard 1989, Staron et al. 2000), and has a higher oxidative capacity than fast twitch fibers. However, studies that have made gender comparisons using this muscle find different average fiber cross sectional areas (males have greater Type II and smaller Type I area) and similar oxidative capacity between genders (Carter et al. 2001, Simoneau and Bouchard 1989). It is unclear what effect differences in muscle fiber type between

genders influenced our study findings or comparative findings. However, given that total lipid use was comparable between genders, it is unlikely that there were differences in oxidative enzymes between groups.

A fourth potential regulator of IMCL use, is fatty acid reesterification once it enters from plasma. Dyck and Bohen (1998) used a pulse chase palmitate labeling technique to study isolated fatty acid release and esterification in skeletal muscle TG (IMCL). Isolated soleus muscle was studied using various continuous tetanic contractions (2, 8, 20, or 40 tetani/min (30 min)). Their results suggested that all fatty acids mobilized from IMCL sources were oxidized, but that approximately 30-35% of fatty acids entering the cell from plasma sources were reesterified back into IMCL, regardless of contraction rate. Thus, only 65% of fatty acids entering from outside the cell were oxidized, and the remainder were reesterified and stored within the cell during exercise. The mechanism for increased TG synthesis was unclear, but the authors suggested it was related to HSL-M control (phosphorylation/dephosphorylation) and acute changes in substrate availability (i.e, increased glycerol and muscle glycerol kinase activity (Guo 1999). Findings of FFA reesterification into IMCL during exercise are similar to what has been reported by others (Hopp and Palmer 1990, Gorski and Bonen 1997, Sidossis et al. 1997). However, no gender studies have been completed. .

These data (35% FFA reeesterification) are in sharp contrast to what has been reported by Romijn et al. (1993), who suggested that exogenous muscle lipid source of fatty acids provide the majority of fuel for muscle during exercise. Romijn et al. (1993) made the assumption that all fatty acid entering the muscle cell were oxidized, but it appears that a large portion are reesterfied and stored during exercise (Dyck and Bohen

1998). Thus, there are opposing ideas with regard to the fate of fatty acids entering the myocyte. If reesterification does occur with exercise, than our post exercise measure would have underestimated the use of IMCL during exercise.

Taken together, these recent studies suggest that IMCL makes a much larger contribution to fuel metabolism during moderate intensity exercise than either low or high intensity exercise. It is also unclear whether there are differences in males and females in regard to fatty acid reesterification. Future studies addressing this question would be helpful in understanding more about lipid metabolism and should focus on gender differences in fatty acid reesterified that enter into the muscle cell.

Given that several factors contribute to FFA mobilization and oxidation during exercise, a lack of control of confounding factors could explain some of the discrepancies noted between our work and other published studies. Differences in pre exercise diet, training status of subjects, difference in exercise interventions, and lack of control of menstrual status could impact lipid metabolism. Our study was designed to control for these confounding factors. For example, women in the luteal phase of the follicular cycle oxidize FFA greater than male subjects at rest because of estrogens ability to increase FFA mobilization (Nathan and Chaudhuri 1997). Several earlier studies made no control of menstrual cycle, and differences between genders could be explained strictly by estrogen fluctuation as opposed to the exercise intervention. The few studies that have had excellent control of the above mentioned factors find little differences in lipid metabolism between genders.

Carbohydrate Results

In addition to examining lipid metabolism, plasma glucose, glucagon, catecholamines, and RER measurements were used to examine gender differences in glucose metabolism. RER data suggested that there were no relative differences in carbohydrate metabolism, but absolute data suggested that male subjects used more carbohydrate than female subjects. Plasma glucose levels were not different between subjects, nor altered with exercise. These results may be a result of increased glucagon and catecholamine levels. Glucagon is involved in mobilizing glucose from the liver for oxidation (Guyton 19991). Plasma glucagon was significantly higher in male subjects following exercise, perhaps helping explain the trend for increased carbohydrate metabolism for male subjects. However, both epinephrine and norepinephrine increased during exercise for both groups, and may have contributed to increased glucose availability through muscle glycogenolysis (Febbraio et al. 1998).

Given that plasma glucose levels were not changed following exercise, it is likely that increased carbohydrate mobilized from liver and muscle sources contributed to maintenance of plasma glucose levels. Our finding of increased glucagon with exercise is similar to what has been reported by others (Tarnolposky et. al 1990).

However, these data are in contrast to what has been reported in gender exercise studies with glucagon (no differences) (Davis et al. 2000, Marliss et al. 2000, Tarnopolsky et al. 1990), and the first to our knowledge to find gender differences in glucagon after exercise. Reasons for differences between genders is unclear, but may increased glucagon in male subjects may have contributed to the increased availability of carbohydrate for male subjects. Further work is warranted in this area.

Summary

In summary, comparisons were made in markers of total body, adipose, plasma, and skeletal muscle lipid metabolism between men and women during a single cycle exercise session. There were no differences in absolute kcal or relative % lipid oxidation during exercise between male and female subjects. These data suggest that overall lipid oxidation did not different between genders. Markers of adipose lipolysis, FFA and glycerol were significantly elevated during, and immediately after exercise in both male and female subjects. Overall group differences were observed for these markers, suggesting greater FFA mobilization for female subjects during exercise. Lipid mobilizing hormones, norepinephrine and GH increased significantly during exercise. Gender differences were observed for norepinephrine, possibly contributing to the increased mobilization of adipose FFA for female subjects and increased glycogenolysis for male subjects. Significant increases were observed for glucagon, a glucose-mobilizing hormone, for both male and female subjects. Male subjects had significantly greater increases in glucagon, potentially contributing to increased carbohydrate oxidation by male subjects. Although not statistically significant, decreases were observed in plasma insulin for both male and female subjects. IMCL decreases were observed for both male and female subjects, although there were no gender differences. Subjects with the greatest baseline IMCL or body fat % decreased IMCL the greatest. Changes in adipose and muscle FFA mobilization may have been from increased activation of HSL-A and HSL-M. However, changes in IMCL were not correlated with catecholamine activity, suggesting that other mechanisms (increased muscle contraction) were involved in activating HSL-M.

Based on the plasma FFA and glycerol responses during exercise, we conclude that female subjects mobilize more FFA from adipose tissue than male subjects during exercise, but that overall lipid oxidation is not different. IMCL is significantly decreased for both male and female subjects during exercise, but our data do not allow a conclusion of gender differences.

Future research should focus on areas that identify gender differences including, 1) ability to mobilize FFA from adipose sites, including how much reesterification occurs during exercise, 2) ability to transport lipid into the muscle cell during exercise, with a particular focus on fatty acid transport proteins, 3) fatty acid reesterification once entry is made into skeletal muscle, 4) ability to form fatty acyl CoA from substrate material, and 5) the ability to take up fatty acyl CoA for oxidation. These comparisons should be made in different fiber types and different states of the menstrual cycle for female subjects. Valuable techniques to aid in the study of these questions include: ^{1}H-MRS, tracer technology, muscle biopsies, metabolic information, and blood samples. Performance of these studies should add valuable research information, given the challenging nature of these research questions.

APPENDIX A
ABBREVIATION AND DEFINITIONS

Carnitine palmitoyltransferase I (CPT) - Enzyme in mitochondria involved in shuttling of fatty acid in for oxidation.

cAMP - cyclic amine phosphate. Phosphate involved in a second messenger pathway.

Chemical Shift Selective (CHESS) - water signal will be suppressed by three consecutive CHESS (Chemical Shift Selective) pulses.

Echo time (ET) - The time between the first RF pulse and the center of the spin echo in a spin echo acquisition.

Extramyocellular lipid (EMCL) - Lipid found in interstitial space between muscle fibers. Thought to be metabolically inactive.

Fatty acid binding protein (FABP) - Protein involved in transport of fatty acid from plasma into cytosol of myocyte.

Free fatty acid (FFA) - Carbon chained lipid used for fuel metabolism. May be stored as a triglyceride.

Free induction decay (FID) - The loss of signal that arises from the randomization of phase coherence following the excitation by an RF pulse.

Hormone Sensitive Lipase (HSL) - Lipase involved in triglyceride hydrolysis.

Intramyocellular lipid (IMCL) - Lipid found in skeletal muscle in close proximity to the mitochondria. Thought to be metabolically active and involved in fuel metabolism.

Long chain fatty acid (LCFA) - Fatty acids with greater than 12 carbons.

Lipoprotein lipase (LPL) - Lipase involved plasma triglyceride hydrolysis.

Point resolved spectroscopy sequence (PRESS) - Sequence used to obtain spectra data.

Proton magnetic resonance spectroscopy (^{1}H-MRS) - Technique used to quantify intramyocellular lipid.

Radiofrequency coil (RF) - Coil used to excite hydrogen nuclei in tissues with pulses of energy.

Respiratory exchange ratio (RER) - Ratio of carbon dioxide produced to oxygen consumed. RER provides information about fuel substrate. Values typically range from 0.70 to 1.0 during steady state exercise conditions. 0.70 suggests fat oxidation, where 1.0 suggests carbohydrate oxidation.

Stimulated echo acquisition mode (STEAM) - Echo acquisition mode when obtaining spectra data.

Ventilatory threshold (VT) - Threshold similar to lactate threshold, where ventilation rises in a non proportional rate compared to oxygen consumption. Thought to reflect the accumulation of blood lactate quicker than lactates removal.

Time domain analysis algorithm (VARPRO) - Algorithm used to curve fit spectra, allowing quantification.

Triglyceride (TG) - Glycerol and 3 fatty acids. The storage form of lipid in adipose and skeletal muscle.

Very low density lipoprotein cholesterol triglyceride (VLDL-TG) - Lipoprotein that transports triglyceride through plasma.

VO_{2max} - Maximal cardiorespiratory fitness.

APPENDIX B
EXERCISE DATA FIGURES

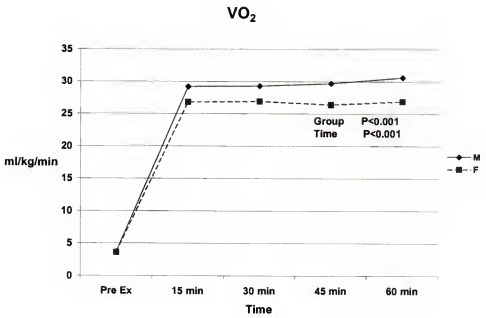


Figure 1. During Exercise VO_2 Responses

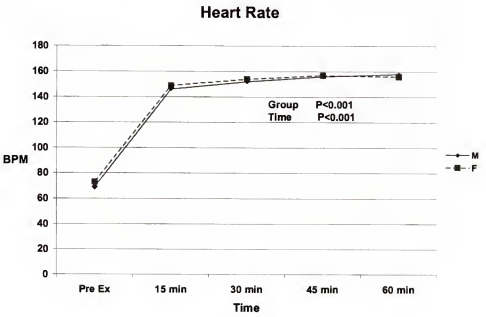


Figure 2. During Exercise Heart Rate Responses

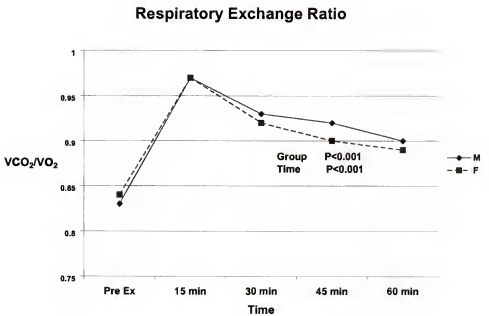
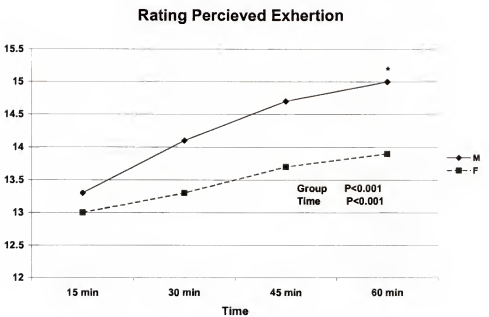


Figure 3. During Exercise RER Responses



*P<0.05, between M and F at 60 min

Figure 4. During Exercise RPE

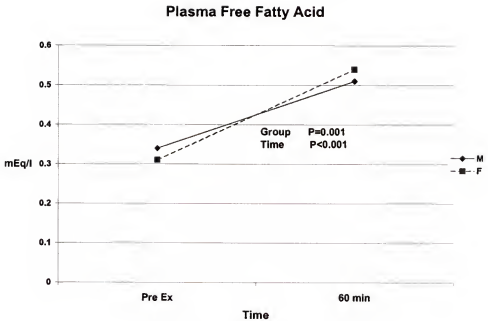


Figure 5. FFA Response to Exercise (plasma volume corrected)

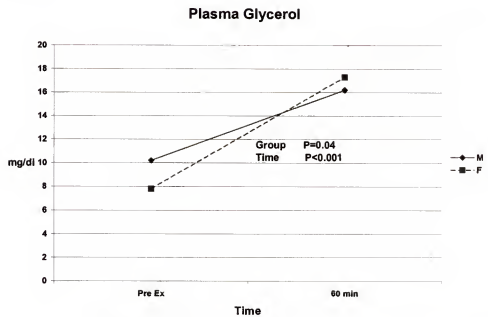


Figure 6. Glycerol Response to Exercise (plasma volume corrected)

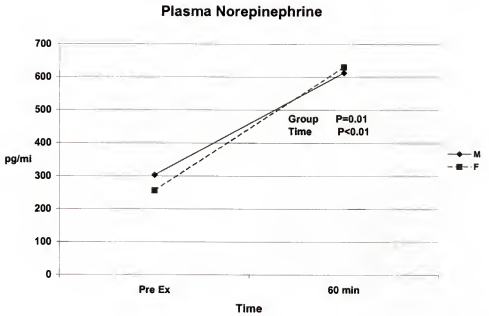


Figure 7. Norepinephrine Response to Exercise (plasma volume corrected)

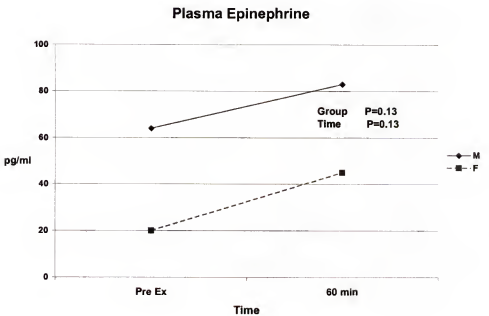


Figure 8. Epinephrine Response to Exercise (plasma volume corrected)

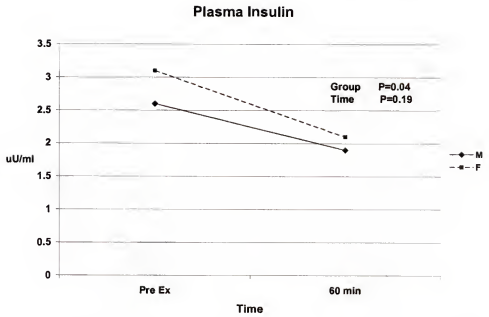


Figure 9. Insulin Response to Exercise (plasma volume corrected)

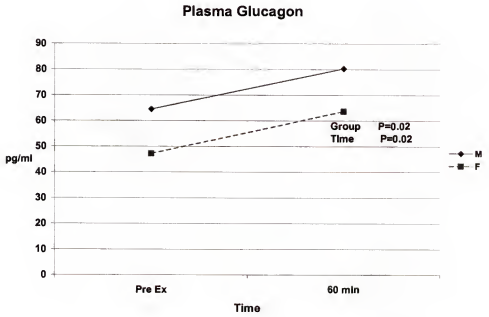


Figure 10. Glucagon Response to Exercise (plasma volume corrected)

APPENDIX C
NMR TECNIQUE

Magnetic Resonance

Theory

Nuclear magnetic resonance spectroscopy (MRS) was developed in the late 1940's and 50's to study nuclei with different spin states (Bruice 1995). In the early 1950's MRS was used to study structures of organic compounds (Bruice 1995). The term nuclear magnetic resonance has traditionally implied proton (^{1}H) MRS since ^{1}H nuclei were the first to be studied. (Some literature refers to nuclear magnetic resonance as NMR). Spectrometers were later developed for ^{13}C MRS, ^{19}F MRS, ^{31}P MRS and other magnetic nuclei. (Bruice 1995). The first clinical applications were performed with animals using small-bore magnets and later with humans using large whole body bore magnets (Boesch 1999). The following is a short summary on the theory of MRS.

Nuclei from specific metabolites can be obtained from unknown compounds or in *vivo* in human tissue (Skeletal muscle, heart, brain, etc.). The above-mentioned nuclei move as though spinning about an axis (Solomons 1992). Nuclei are normally oriented and behave like a bar magnet. This suggests the nuclei have a magnetic moment, or coincide with the axis of the orbital spin. When the sample or tissue of interest is placed in a strong magnetic field and irradated with electromagnetic energy in the radio frequency region (RF) the nuclei absorb energy. The absorption of energy is considered quantized. Nuclei with spins of 1/2, orient themselves in the same direction as the applied magnet (with the external magnetic field), and nuclei with spins at -1/2 orient themselves opposite to that of the magnet (against the external magnetic field). The nuclei aligned with the magnetic field (the alpha spin state) are lower in energy than those aligned against the field (the beta spin state). Although, there is an unequal

distribution of alpha and beta spin states (i.e., only 10-20 protons per million ^{1}H protons in the lower energy state, alpha spin state) this is enough to form the basis of MRS (Bruice 1995).

When electromagnetic radiation is applied, nuclei in the alpha spin state will absorb radiation, which causes them to flip and thus orient the beta spin state. As nuclei relax back to their natural spin states a spectrum is detectable. The energy difference between the alpha and beta spin state is termed $\triangle E$. When the molecules relax and return to its original spin state, energy is released as heat (Bruice 1995). This step is represented by a free induction decay (FID).

The term resonance comes from the fact that nuclei are in resonance, or flipping back and forth between spin states. The energy difference between the alpha and beta spin states is dependent on the field strength of the external magnet field (B_0). The equation below describes the relationship between the energy difference of the alpha spin state and beta spin state and the strength of the magnetic field (B_0) (Solomons 1992).

$$\triangle E = hv$$

$$\triangle E = \frac{hyB_0}{2\pi}$$

$\triangle E$ = energy difference between the alpha and beta spin state, h = Planck's constant, v = frequency, y = magnetic ratio (in radians tesla/sec), B_0 = strength of magnetic field (measured in Tesla (T)). Common field strengths of magnets are 1.5T, 2.1T, 3.0T, and 7.0 T. 1T = 10,000 Gauss. When expressed relative to the earths magnetic field, 1.9T = 38,000 times that of the earths magnetic field (Robergs and Roberts 1997).

MRS spectrometers designed for tissue, irradiate nuclei with electromagnetic energy with short pulses of RF radiation, while the magnetic field strength is varied (Solomons 1992). The RF pulses excite all the nuclei of interest at once, as opposed to each nuclei being individually excited as in traditional methods. The most common MRS spectrometers operate at 60, 100, 200, 300, 360 and 500 MHz (Bruice 1995). This frequency is referred to as the operating frequency and some MRS spectrometers operate as high as 1000 MHz. The greater the operating frequency and stronger the magnet, the greater the resolution of the MRS spectrum (Bruice 1995). The field strength is dependent on the magnetic environment of each proton. The magnetic environment is affected by the magnetic fields generated by circulating electrons and magnetic fields that result from other nearby protons (Solomons 1992). Magnetic field strength is measured along the bottom of the spectrum on a delta scale in units of parts per million (Solomons 1992).

Data are collected as a function of time. Complete spectrums can be generated in as little as 5 s. Since spectral peak data are collected simultaneously, the signal or FID must be transformed into the frequency domain through a mathematical Fourier transformation (Solomons 1992). Resulting spectra occurring at high magnetic field strengths (upfield) have small delta values, whereas those occurring at low magnetic field strengths (downfield) have large delta values. Height of spectra, when integrated, represents the relative number of protons and not absolute number. Quantification of spectra includes expression of metabolite data as a percent of water or creatine content, arbitrary units (au) or area under the curve, or as mmol of weight per unit of muscle (Kreis 1997).

Application of ^1H Acquisition

There are several steps for obtaining spectra. Regardless of the magnet strength used, the following procedures are applicable to clinical use. In metabolism studies, several skeletal muscles can be evaluated. These include the vastus lateralis, gastrocnemius, and soleus muscles. In other clinical studies it is typical to use the heart, brain, and liver.

After placing a subject in the magnet and marking the scanning region (i.e., with a marking pen) it is important to the identify a region of interest (ROI). The optimal ROI represents entire muscle morphology. As the ROI is identified the procedure is synonymous to obtaining a magnetic resonance image. Once the image is obtained in the ROI it is imperative to identify the voxel (single region of interest, typically 2 x 2 x 2 cm^3) site.

The protocols that work best for single voxel scanning are the point resolved spectroscopy sequence (PRESS) and stimulated echo acquisition mode (STEAM) sequences (Kreis 1997). These protocols have the best reproducibility. For multi voxel scanning the chemical shift imaging (CSI) technique is usually chosen (Kreis 1997). In most ^{1}H-MRS metabolism studies single voxel scanning is used.

An advantage of the PRESS sequence is that it has less signal to noise ratio than the STEAM sequence, but the STEAM sequence is more robust. The STEAM sequence is also less demanding in terms of peak RF power (Kreis 1997).

Another important step is the optimization of echo time (ET). Echo time is the time between the first RF pulse and the center of the spin echo acquisition (White 1999). A short ET gives rise to the smallest susceptibility to T_2 loss (changes due to orientation

of scanned region (Chu et al. 1990)). However, background signals are greater with short ET allowing greater contamination from outer volume signals. An ET of 20-30 ms maximizes spectra obtainment (Kreis 1997).

Another important aspect of MRS spectroscopy is the suppression of the water signal. Because H_20 makes up a large portion of skeletal muscle, water peaks are very tall and must be suppressed. This allows better visualization of smaller lipid peaks. Water suppression using the PRESS or STEAM sequences is completed through a process known as presaturation. Presaturation uses multiple chemical shift RF pulses with narrow bandwidths (normally 50-100Hz at 1.5T), followed by gradient dephasing to suppress the water signal.

To further enhance picture quality, a shimming step is included. Shimming enhances spectra quality through fine-tuning of spectra and can be done manually or automatically. Automated shimming is faster than manual shimming, but may be less sensitive to fine adjustments (Kreis 1997). In the proposed study shimming will be performed manually to ensure quality spectra resolution.

Once data is obtained, several additional steps must occur before data processing can occur. These include: amplitude, phase, or line shape corrections based on reference scan.

Following baseline correction, data is processed with the appropriate transformation (Fourier) model. Much debate exists as to the use of either frequency or time domain fitting. When used appropriately, both techniques produce similar results. The frequency domain is the most common line fitting approach for ^{1}H-MRS. The Marquardt-Levenberg algorithm is an example of a common frequency domain (Kreis

1997). Following application of the above-mentioned fitting routine, resonance lines usually approximate Lorentzian or Gaussian shape. It is common to use prior knowledge of resonance patterns to eliminate errors when applying line fits, especially when measuring multiple peaks (Kreis 1997). Gaussian shapes are typical for evaluating muscle lipid.

When using a time domain analysis algorithm (VARPRO), nonlinear least squares fitting algorithms, can accommodate prior knowledge. However, this program needs starting values, usually obtained from prior knowledge, i.e. peak picking in the frequency domain.

The last step of MRS is to express metabolite data. Several methods have been used. However, there is no consensus. These include: single metabolite as internal standard, using an external reference standard, using water as internal standard, reporting total area under curve or AU, or expression as a unit of tissue weight (Kreis 1997).

In summary, MRS is a technique that allows quantification of metabolites in *vivo* and in *vitro*. This technique uses the principle that nuclei are excitable. By examining the rates of energized nuclei decay over time and the resultant spectra quantification is possible. Over time this technique will gain continued favor for clinical pathology and preventive medicine.

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APPENDIX D
POWER CALCULATION

Decombaz et al. 2001 (N=6)

Pre Exercise IMCL measure: 3.34 $\pm$ 0.53 (mmol/kg ww $\pm$ SD) Post Exercise IMCL 2.53 $\pm$ 0.43

n per group = $2[(Z\alpha - Z\beta)\sigma/(u_1-u_2)]^2$

$Z\alpha$ = alpha level for two tailed Z
$Z\beta$ = lower one-tailed Z value that is related to β
σ = maximal variance
u_1-u_2 = difference between mean 1 and 2

$n = 2[(1.96 + 0.84)(0.53)/(0.81)]^2$

$= 2(3.35) = 6.7$ subjects
so, 7 subjects per group

Power = 0.80

Decombaz J., Schmitt B., Ith M., Decarli B., Diem P., Kreis R., Hoppeler H., Boesch C. (2001). Postexercise fat intake repletes intramyocellular lipids but not faster in trained than in sedentary subjects. Am. J. Phys. 281: R760-R769.

APPENDIX E
HEALTH RISK ASSESSMENT

Health Risk Questionnaire

Date_____

Name _____ Phone Number _____

Address _____

Male Female Date of Birth _____

Ethnicity: Caucasian African American Hispanic Asian Other

Emergency Contact and Number _____

Family Physician and Number _____

Please circle any of the following that apply.

1. Have you been diagnosed with diabetes? Yes No

If yes, please explain.

2. Have you ever had an oral glucose tolerance test? Yes No

If yes, when?_____

3. Have you ever been told you had high blood pressure? Yes No

If yes, when and are you on medication? _____

4. Do you have high cholesterol levels? Yes No

If yes, are you on medication? _____

5. Do you exercise (at least 30 minutes) at least 3-5 times per week? Yes No

6. Do you smoke cigarettes or cigars or chew tobacco? Yes No

If yes, how often and how much? _____

7. Do you drink alcoholic beverages? Yes No

If yes, how often and how much? _____

8. Do you consider most of your days very stressful? Yes No

9. Do you consider your eating habits healthy overall? Yes No Moderate
(Lower in fats and fried foods, higher in fruits, veggies, and grains)

10. Have you ever had shortness of breath or chest discomfort during exertion?

Yes No

If yes, please explain. _____

11. Is there any history of heart or cardiovascular problems in your family?

Yes No

If yes, please explain_____

12. Have you had any major surgeries? Yes No

If yes, please explain._____

13. Please list any medications you are currently taking that are not listed on the above questions.

14. Do you have any orthopedic limitations that would prevent you from participating in vigorous activity? Yes No

If yes, please explain. _____

15. Do you have a heart valve or implant devices such as knee, hip, etc?

Yes No

If yes, please explain. _____

16. Do you get claustrophobic in small spaces? Yes No

17. Has your weight changed more than 5 pounds in the last 6 months? Yes No

If yes, please explain. _____

18. Have you had any of the following? Bone growth stimulator, ear prosthesis, magnetic dental implants, brain aneurysm clip, shrapnel or bullet injury. Yes No

If yes, please explain. _____

APPENDIX F
PHYSICAL ACTIVITY STATUS

Physical Activity Questionnaire

Name_____ Date_____

Please respond to the following questions by circling the most appropriate answer.

1. Are you currently participating in sustained physical activity (at least 30 minutes per session) at least 3-5 times per week? Yes No

If yes, what types of activities are you doing? _____

How intense is this activity usually? Very Hard Hard Moderate Easy

2. Have you participated in regular physical activity (at least 30 minutes per session) at least 3-5 times per week in the last 6 months? Yes No

If yes, what type of activities were you doing? _____

How intense was this activity usually? Very Hard Hard Moderate Easy

3. How much time per day and week approximately do you spend in non-sustained exercise physical activity? (example: yardwork, housework, walking to class, grocery shopping, etc.)

Day (minutes) _____ Week _____

4. Do you have a competitive background in athletics? Yes No

If yes, please describe _____

5. What type of snack or drink would you prefer after the 60-minute bike session?

APPENDIX G
DIETARY ASESSMENT

Dietary Diary

Name_____ **Date**_____

Study ID# _____

Please record what you eat and drink for the entire day. Please be as specific as possible for serving size, how food is prepared and number of servings.

Example: 4 ounces grilled chicken breast, 1 cup corn, 1 tsp. butter, 1 piece of white bread, 8 ounces skim milk, etc.

Name of food (cookie, cake, etc.)	Servings (1 cup, 4 ounces, etc.)	How prepared (baked, fried, etc.)
Breakfast		
Lunch		
Dinner		
Snacks		

APPENDIX H
MAXIMAL FITNESS

Maximal VO_2 Bike Test

Name_____ Date_____

Age_____ Predicted Maximal Heart Rate_____

RPE	Workload			Time	HR	BP
Rest	0	Watts		0:00		
Stage 1	50	Watts	(1.0 kp)	0:00 - 1:00		
Stage 2	75	Watts	(1.5 kp)	1:00 - 2:00		
Stage 3	100	Watts	(2.0 kp)	2:00 - 3:00		
Stage 4	125	Watts	(2.5 kp)	3:00 - 4:00		
Stage 5	150	Watts	(3.0 kp)	4:00 - 5:00		
Stage 6	175	Watts	(3.5 kp)	5:00 - 6:00		
Stage 7	200	Watts	(4.0 kp)	6:00 - 7:00		
Stage 8	225	Watts	(4.5 kp)	7:00 - 8:00		
Stage 9	250	Watts	(5.0 kp)	8:00 - 9:00		
Stage 10	275	Watts	(5.5 kp)	9:00 - 10:00		
Stage 11	300	Watts	(6.0 kp)	10:00 - 11:00		
Stage 12	325	Watts	(6.5 kp)	11:00 - 12:00		
Stage 13	350	Watts	(7.0 kp)	12:00 - 13:00		
Stage 14	375	Watts	(7.5 kp)	13:00 - 14:00		

Exercise Test Time_____ Maximal Heart Rate Achieved_____

65% VO_2_____ VO_2 Range_____ Heart Rate at 65% VO_2_____
(± 5%)

APPENDIX I
EXERCISE DATA COLLECTION

During Exercise Data Collection

Pre Exercise

Name_____ Date _____

Weight _____ VO_2 (65% ± 5%) _____

Heart Rate Range _____ Workload _____

Water Volume Amount Per 30 Min Period _____

Hematocrit _____ Hemoglobin _____

Resting VO_2 _____ ml/kg/min RER _____

During Exercise

VO_2 (1-3 min) _____ ml/kg/min RER _____

VO_2 (8-10 min) _____ ml/kg/min RER _____

VO_2 (28-30 min) _____ ml/kg/min RER _____

Hematocrit _____ Hemoglobin _____

Water Intake _____ RPE _____

VO_2 (43-45 min) _____ ml/kg/min RER _____

Hematocrit _____ Hemoglobin _____

VO_2 (58-60 min) _____ ml/kg/min RER _____

Hematocrit _____ Hemoglobin _____

Water Intake _____ RPE _____

Post Exercise

Hematocrit _____ Hemoglobin _____

Total Water Intake _____ Weight _____

APPENDIX J
MENSTRUAL INFORMATION

Menstrual History

1. Have you had regular menstrual periods over the last 6 months? Yes No

2. When did you have your last menstrual period? _____

3. Are you taking or on any form of birth control? Yes No

If yes, please describe? _____

4. What is the date of the estimated first day of your next period? _____

5. Are you currently pregnant? Yes No

6. Estimated date range for testing during early to mid follicular phase _____

7. Scheduled test date _____

APPENDIX K
CONSENT

IRB# 5-00

Informed Consent to Participate in Research

The University of Florida
Health Science Center
Gainesville, Florida 32610

You are being asked to participate in a research study. This form provides you with information about the study. The Principal Investigator (the person in charge of this research) or his/her representative will also describe this study to you and answer all of your questions. Read the information below and ask questions about anything you don't understand before deciding whether or not to take part. Your participation is entirely voluntary and you can refuse to participate without penalty or loss of benefits to which you are otherwise entitled.

Name of the Subject

Title of Research Study

Influence of Gender on Skeletal Muscle Metabolism During Exercise: A Proton Magnetic Resonance (^{1}H-MRS) Study

Principal Investigator(s) and Telephone Number(s)

Lesley J. White, Ph.D. (352) 392-9575 ext. 1338
Department of Exercise and Sport Sciences
University of Florida
27 Florida Gym

Katherine Scott, Ph.D. (352) 376-1611 ext. 5066
Department of Radiology
College of Medicine
University of Florida

Sponsor of the Study

University of Florida

What is the purpose of this study?

The purpose of this investigation is to examine the effects of exercise on the utilization of fat stored within skeletal muscle. This study is part of a project to learn more about energy production during exercise. Our plan is to get pictures of your leg muscles using a technique called magnetic resonance imaging (MRI). Then we can get information about the chemistry of your muscle using a technique called magnetic resonance spectroscopy (MRS).

What will be done if you take part in this research study?

If you volunteer to participate in this study, the following things will happen: you will be asked to perform several tests. These tests will estimate your percent body fat, measure your maximal ability to cycle or run on a treadmill, and determine the quality of your diet. Treadmill testing and percent body fat assessment will take approximately 60 minutes to complete. These tests will be completed in the Center for Exercise and Sport Sciences in the Florida Gym located on the main campus of the University of Florida.

At another time, you will lie on a bed, which rolls in the opening of a large magnet. A flat coil of wire (a radiofrequency coil) will be placed on your thigh. A computer looks at the radio waves passing through your heart and constructs pictures and chemical information of your muscles. The total procedure will last approximately 30 minutes. With the aid of additional computers, this instrument will measure the fat content in the muscles of your leg. You will then ride a stationary bicycle or run on a treadmill for 45 to 90 minutes, and after this time, you will once again enter into the large instrument and magnetic field. The instrument is harmless to the body, and is used routinely to detect structures or gain chemical information about the muscles of healthy subjects or hospital patients. Before exercise, a needle with a plastic tube ("catheter") attached will be inserted into your arm by a trained phlebotomist. This will allow us to take blood samples before, during, and after your exercise to measure the level of fat, sugar, and insulin in your blood. Insulin is a substance that the body needs to use sugar. The total amount of blood we will take will be less than 10 tablespoons.

What are the possible discomforts and risks?

There are some risks to your health and well being if you agree to be a subject in this research. Dr. Lesley White will discuss these with you. The risks of these tests are as follows. The body fat test requires you to be submerged in a tank full of warmed water containing bromide. According to the National Pool and Spa Association, the incidence of skin irritation to bromide in water is <0.001%. To make the test of body fat more accurate, you will be required to also breathe into a tube and instrument that will measure the volume of air that remains in your lungs after a full expiration. The test of my maximal cycling or running ability will be completed on a treadmill or cycle ergometer, respectively. This test will require you to run or cycle at increasing exercise intensities over a 15-minute period until you can no longer continue to exercise. During this test, you will also breathe through a valve and have my expired air analyzed by an instrument

that will calculate the amount of oxygen my body uses during the test. According to the American College of Sports Medicine, the risks of completing such a test include sudden death, unusual heartbeats, and heart attack. However, the incidence of these events in even elderly and unhealthy people is less than 0.01%. In fact, you are exposed to similar risks when you exercise to fatigue during recreational activities. Should any problems occur during testing, the personnel testing you are trained in cardiopulmonary resuscitation, and emergency procedures exist for contacting medical support as soon as possible. The test evaluating your diet is simply a questionnaire that will require you to list the foods and their quantities after you consume them over a three-day period.

The risks associated with MRI and MRS is minimal. However, if you have any type of metal implanted into your body, you may not be able to have the MRI/MRS. Someone will ask you questions before you have the MRI/MRS. You will not be able to have the study if you have any pacing devices (such as a heart pacemaker), any metal in your eyes, any brain aneurysm clips, or certain types of heart valves. Likewise, metal objects such as coins, glasses, hairpins, jewelry, and mascara should not be present when you go into the magnet.

The minimal risks of a strong scanner are similar to those of the conventional MR scanners. The radio waves may or may not be stronger than those for a conventional MRI scanner. We keep the heating effects within the guidelines of the FDA. But there is a small chance that the controls would fail, and you may experience localized heating to your skin. All of the controls used on the scanner and in this study have been specifically designed to prevent this from occurring. However, if you feel any localized heating sensation, simply tell the operator, and the scan will be stopped immediately.

In addition, the MR scanner produces a "Hammering" noise, which has been reported to have produced hearing loss in a very small number of patients. You will be given earplugs to further reduce this risk. You will be monitored during the entire study.

The 45-90 minute cycle or run will be completed at a pace that is comfortable. You will be allowed to drink chilled water whenever you want, and a fan will be used to aid in the transfer of heat from your body. The risk of leg muscle soreness for three days following this exercise test are high (50%), however the soreness should not restrict your ability to perform your daily activities.

The risks of drawing blood from a vein include discomfort at the site of puncture, possible bruising and swelling around the puncture site, rarely an infection and uncommonly, faintness from the procedure. The risk from having a catheter inserted into an arm vein for time needed in this study is possible infection of the vein, but the risk is very low because we will have a trained phlebotomist inserting the catheter. The amount of blood we will take should have no negative effect. You will be closely watched for any adverse effects.

What are the possible benefits to you or to others?

There may be some benefits to you for agreeing to participate in this investigation. Results from the assessments of percent body fat, maximum oxygen consumption, blood sugar levels, and muscle lipid content will be made available to you. In addition, the quantity and quality of your diet assessed over a three-day evaluation period will also be available to you.

If you choose to take part in this study, will it cost you anything?

All costs associated with the assessment of your percent body fat, maximal oxygen consumption and quality of your diet will be paid for by the Center for Exercise and Sport Sciences. In addition, the principle investigator's funding will pay for the costs associated with the measurement of the fat content in your muscle. There will be no cost to you for your participation in this investigation.

Will you receive compensation for your participation in this study?

You will not receive any financial compensation for your participation in this research.

What if you are injured because of the study?

If you experience an injury that is directly caused by this study, only professional medical that you receive at the University of Florida Health Science Center will be provided without charge. However, hospital expenses will have to be paid by you or your insurance provider. No other compensation is offered.

If you do not want to take part in this study, what other options or treatments are available to you?

Participation in this study is entirely voluntary. You are free to refuse to be in the study, and your refusal will not influence current or future health care you receive at this institution.

How can you withdraw from this research study?

If you wish to stop your participation in this research study for any reason, you should contact: Dr. Lesley White at (352) 392-9575 ext. 338. You are free to withdraw your consent and stop participation in this research study at any time without penalty or loss of benefits to which you are otherwise entitled. Throughout the study, the researchers will notify you of new information that may become available and that might affect your decision to remain in the study.

In addition, if you have any questions regarding your rights as a research subject, you may phone the Institutional Review Board (IRB) office at (352) 846-1494.

How will your privacy and the confidentiality of your research records be protected?

Authorized persons from the University of Florida, the hospital or clinic (if any) involved in this research, and the Institutional Review Board have the legal right to review your research records and will protect the confidentiality of those records to the extent permitted by law. If the research project is sponsored or if it is being conducted under the authority of the United States Food and Drug Administration (FDA), then the sponsor, the sponsor's agent, and the FDA also have the legal right to review your research records. Otherwise, your research records will not be released without your consent unless required by law or a court order.

If the results of this research are published or presented at scientific meetings, your identity will not be disclosed.

Will the researchers benefit from your participation in this study (beyond publishing Or presenting the results)?

The researchers will not benefit from your participation in this study beyond the professional benefit from the academic publication or presentation of the results.

Signatures

As a representative of this study, I have explained the purpose, the procedures, the benefits, and the risks that are involved in this research study:

_____ _____

Signature of person obtaining consent Date

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this Form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

_____ _____

Signature of Subject Date

_____ _____

Signature of Witness (if available) Date

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BIOGRAPHICAL SKETCH

From 1988 to 1992 I attended the University Tennessee Chattanooga on athletic and academic scholarships. I graduated with a B.S. in Exercise Science, under the tutelage of Dr. Patricia Mosher. In 1992 I began work toward a M.S. degree in Exercise Science at the University of South Carolina with Dr. Larry Durstine. Following graduation I worked as a Research Assistant in the Department of Pediatrics with Dr. Bob Gutin. During this time I was accepted as a Ph.D. student at the University of Florida with Dr. Scott Powers. Two years into the program I switched advisors to Dr. Lesley White. My graduation occurred in 2002, approximately 15 years after beginning my academic career. During the later portion of my Ph.D. work I decided to pursue industry as opposed to academic work. This seemed a logical choice given the given the greater financial incentives and greater time available to pursue non-academic interests.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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August 2002

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